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The chemical ecology, genetics and impact of the European earwig in apple and cherry orchards

by

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Submitted in fulfilment of the requirements for the degree of Doctorate of Philosophy

University of Tasmania August 2013

Declaration

I hereby declare that this thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Stephen Quarrell

Abstract

This thesis investigates the Australian distribution, invasion biology and genetics of the European earwig, *Forficula auricularia*, its predation of woolly apple aphid (WAA) and intraguild compatibility with the parasitoid *Aphelinus mali* in apple orchards, the impact earwigs have upon sweet cherry production and the chemical ecology of *F. auricularia* with special reference to the isolation of its aggregation pheromone.

F. auricularia was found to be spread across all of southern Australia with records indicating it probably invaded Australia, in Tasmania, over 170 years ago. The mtDNA analysis of Australian and New Zealand *F. auricularia* populations indicated only one of the two known European earwig subspecies is found in these regions and that there are two differing clades of this subspecies within Australia but only one in Tasmania and New Zealand. Comparing these results to samples collected throughout Europe indicates that the genetic diversity of the mainland Australian population is only half that of Europe and the diversity in Tasmania and New Zealand is half that again. Possible European sources for only one of the two Australian clades were found. These results indicate that multiple invasion events are likely to have occurred on the Australian mainland, but this seems less probable within Tasmania or New Zealand.

The investigation into the intraguild compatibility of earwigs and *A. mali* in apple orchards was determined by weekly monitoring of arthropod communities (including WAA, earwigs, *A .mali*) within 5 orchards over two entire apple production seasons. Earwig trap catches were observed to rapidly decline after the imaginal moult at all sites and during both seasons. The thesis shows that trees which possess large earwig trap catches (> 22 earwigs/tree/week) within the first 7 weeks after blossom contain little to no WAA at the end of the season. Trees that contained fewer earwigs had larger WAA infestations unless the first generation of *A. mali* numbers exceeded 0.5 wasps per sticky trap per week. If these beneficial insect targets were not met, extreme WAA infestations occurred, despite other predators being observed feeding on WAA colonies.

Cherry fruit and cherry stem damage assessments were conducted on four commercial cherry varieties; Ron's Seedling, Lewis, Sweet Georgia and Lapin. Assessments of the spatial distribution of earwigs within cherry canopies and the cherry bunch characteristics including

bunch size and position, and the level of cherry fruit or cherry stem damage that may have ensued were determined. Significant differences in the type and frequency of earwig damage were observed between varieties with damage varying between 5-60%. Earwigs were found to be strongly aggregated within large cherry bunches. The greatest damage was observed within these large bunches in all varieties except Ron's Seedling where stem damage occurred irrelevant of bunch size. No predictive relationship between the level of cherry damage and earwig numbers in trunk traps at harvest or those found within the tree canopies at harvest could be found.

Chemical ecology experiments demonstrated earwigs were attracted to substrates pre-exposed to earwigs in both laboratory and field bioassays. The thesis newly identifies numerous headspace volatiles and cuticular hydrocarbons (HC) isolated from aggregating male, female and juvenile earwigs. Some promising synthetic blends consisting of unsaturated HCs demonstrated earwig attraction twice that of controls in the field. However, attraction to these blends was inconsistent across the earwig life cycle and field season. To investigate whether the observed decline in earwig trap catches and the inconsistent attraction to the synthetic pheromone blends was due to pheromone plasticity, sequential sampling of earwig populations while simultaneously sampling the cuticular HCs from the same field populations was undertaken. Results demonstrated that the production of cuticular HCs in *F. auricularia* decline soon after the imaginal moult and that this decline correlates with a decline in earwig trap catches. Although promising aggregating compounds have now been identified, further work, especially on the consistency of their bioactivity is needed.

Acknowledgments

Firstly, I would like to thank my supervisors, Geoff Allen, Paul Walker and Noel Davies whose help and advice has been priceless.

I also wish to acknowledge Horticulture Australia Limited and the Holsworth Wildlife Research Endowment for their financial support.

I also thank the following people:

Ross Corkrey for his assistance and tutelage in the statistical analysis used in most of the aspects of this work.

Jason Smith for the synthesis of the unsaturated hydrocarbons used in the bioassays.

Thierry Wirth, Juliette Arabi and Alice Balard from the Muséum National d'Histoire Naturelle – EPHE for their assistance with the genetic analysis and their hospitality during my visit, I will be forever grateful.

The apple and cherry producers John Evans, Andrew Smith, Simon Burgess, Scott Coupland, Howard Hansen, Ross Kile and Robert Fitzpatrick who graciously provided their time and resources and Peter Kennedy from Delta Agribusiness in Young, NSW whose local knowledge helped locate sites in the NSW area.

Thanks also to Mélusine Lefebvre, Shasta Jamieson, Peter Lehman, Gemma Bilac, Bianca Deans, Cathy Byrne, Peter McQuillan, Charles Melton, Jamie Davies, Gerry Cassis, Peggy Quarrell, Svetlana Micic, Marc Widmer, Toni Withers, David Rentz, Laurie Parkinson, Alistair Gracie, Sally Jones, Robert Brockman, Alicia Tracey, and Chantal Woodhams all of whom have helped in some form over the project.

Finally, I wish to thank my wife Nicole, for her support, patience and assistance on countless occasions, especially on weekends when I am sure she would have preferred to be doing something other than counting earwigs.

Preface

In this thesis, each experimental chapter (chapters 2 - 6) has been prepared in the form of a publishable manuscript with the references placed at the end of each chapter, which have been formatted for their target journal as indicated on the front page of each chapter. Tables and figures have been re-labelled to fit within each chapter. Due to this independence between chapters there maybe overlap or repetition within this thesis. This thesis has been divided into seven chapters. Chapter 1 is a general introduction that reviews European earwig phenology, its use as a biological control agent in apple orchards and its chemical ecology with a focus on previous attempts to isolate its aggregation pheromone. Chapter 2 investigates the current Australian distribution and genetic diversity of *F. auricularia* and attempts to identify the overseas source of its accidental introduction into Australia and New Zealand. Chapter 3 examines the efficacy of earwigs as biological control agents in apple orchards against the woolly apple aphid, *Eriosoma lanigerum* (WAA) and further examines how earwigs and the WAA parasitoid, *Aphelinus mali* interact to suppress WAA numbers below problematic levels. Chapter 4 explores the ecology of European earwigs in cherry orchards and examines their spatial distribution within cherry tree canopies and the potential impact this has on cherry fruit and cherry stem damage. Chapter 5 identifies putative aggregation pheromone components emitted by *F. auricularia* and assesses these compounds for behavioural activity. Chapter 6 investigates the phenology of the cuticular hydrocarbon profiles of *F. auricularia* and how these fluctuations may relate to earwig population dynamics. Finally, Chapter 7 is a general discussion, which integrates the findings from chapter 2 to 6 and makes recommendations as to further research.

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Chapter 1 Introduction

The European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) is a cosmopolitan insect species found in many temperate regions. It is endemic to Europe, western Asia and possibly northern Africa (Lamb & Wellington 1975). However, accidental introductions into many countries in both northern and southern hemispheres have resulted in successfully established populations worldwide (Rentz & Kevan 1991). European earwigs were first discovered in Tasmania prior to 1903 (Lea 1903) and in 1930 on the Australian mainland outside Sydney (Gurney 1934). In 1994 this species was first discovered near Albany, Western Australia and has since continued its spread into Western Australia's south-west (Widmer *et al.* 2008).

Several studies have shown that *F. auricularia* aggregate in large numbers with the use of an aggregation pheromone (Hehar 2007; Sauphanor 1992; Walker *et al.* 1993). This coupled with an omnivorous feeding habit has led it to being considered both an urban (Lamb & Wellington 1975; Walker *et al.* 1993) and agricultural pest in many vegetable (Rentz & Kevan 1991) and soft-fleshed fruit crops such as raspberries (Gordon *et al.* 1997), cherries, apricots, peaches and nectarines (Suckling *et al.* 2006). However, earwigs have also been shown to be a beneficial insect in hop gardens (Buxton & Madge 1977) and apple, citrus (Piñol *et al.* 2012; Piñol *et al.* 2010) and kiwifruit (Logan *et al.* 2011) orchards due to the consumption of various pest insect species including aphids and Lepidopteran larvae (Carroll & Hoyt 1984; Mueller *et al.* 1988; Nicholas *et al.* 2005; Solomon *et al.* 2000; Suckling *et al.* 2006).

Morphology and taxonomy

Adult *F. auricularia* are dorsally flattened, elongate, 15-25 mm in length with males generally larger than females. Their cuticle is smooth to shiny and brown in colour, they bear mandibulate mouthparts, filiform antenna and possess depressed, basally dilated forceps extending from the tip of the abdomen. Adults are winged with a membranous, ear-shaped hindwings, which fold complexly to aid protection beneath the hardened forewing. Juveniles have four instars and resemble adults with wing pads appearing in the 2nd instar (Rentz & Kevan 1991).

F. auricularia are a sexually dimorphic species (Figure 1-1). Males have 10 tergites; females have eight visible with T8 and T9 strongly reduced and fused to T10. Male forceps are heavy

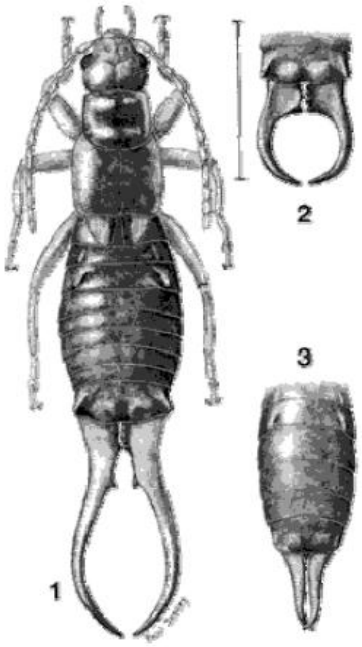

Figure 1-1. European earwig morphology (1) Whole macrolabic male *Forficula auricularia*, (2) tip of abdomen of microlabic male with short, sharply curved forceps, (3) tip of abdomen of female earwig (Weems & Skelley 2007)

and curved with female forceps slender and straighter than the males (Rentz & Kevan 1991; Walker & Fell 2001). Dimorphism occurs amongst males with macrolabic males having long forceps and brachylabic males having shorter forceps with a stronger curvature. Macrolabic males enjoy greater mating success due to an increased competitive ability between males (Walker & Fell 2001) and female preference (Tomkins & Simmons 1998).

Previously, climate and locality were believed to affect *F. auricularia* life-history. High altitude populations were observed laying one clutch per season during early winter with a long gregarious adult phase and no diapause. Those at lower altitudes laid two clutches per season with an imaginal overwintering diapause (Guillet *et al.* 2000), the first clutch being laid at the beginning or end of winter with a second smaller clutch in late spring early summer (Lamb & Wellington 1975). However, more recent genetic analysis of populations in Europe and North America identified two subspecies with differing altitude preferences, subspecies A (one or two clutches per year) residing in the alpine zone >1100 m, and subspecies B (two clutches per year) residing between sea level and 1200 m (Guillet *et al.* 2000). Analysis of a 623 bp mtDNA fragment overlapping Cytochrome oxidase I (COI) and COII identified that interspecific genetic divergence was five to seven times greater than the intraspecific variation. Studies have shown these populations co-exist at an altitude of approximately 1200 m with no sexual interaction apparent between subspecies in the wild (Guillet *et al.* 2000). Forced copulations between subspecies in laboratory experiments showed egg infertility prohibited any genetic flow occurring between subspecies (Wirth *et al.* 1998). DNA analysis of Australian *F. auricularia* populations have yet to be undertaken, therefore the subspeciation in Australian populations is currently unknown.

Recently, laboratory-based mating trials utilising progeny from a combination of one and two clutch females claimed that the two subspecies were a single species with the females choosing differing reproductive strategies dependent on their condition and food availability (Meunier *et al.* 2012). However, as subspecies A is known to produce either one or two

clutches per year (Wirth *et al.* 1998) and genetic analysis of the parental lines was not conducted as was done in similar mating trials conducted by Wirth *et al*. (1998), this assertion remains unfounded.

Biology and lifecycle

In late autumn, male and female earwigs form pairs and excavate subterranean nests > 2 cm beneath the soil surface or under rocks and logs in preparation for overwintering. Nests may have one or more entrances and chambers (Lamb & Wellington 1975). Mating occurs in early autumn (Lamb 1976) and continues through the overwintering phase (S. Quarrell, pers. obs.). Multiple mating has been observed in laboratory experiments but it remains unclear whether this occurs in field populations (Lamb 1976; Walker & Fell 2001). As mating may occur prior to nesting the nesting male may not be the contributor of the paternal line. Brown (2006) postulated that the final matings within the nest may force the sperm from previous matings either out of the spermatheca or to the distal end of the spermatheca where egg fertilisation is reduced. Following nest formation and mating the male exhibits mate guarding behaviours to prevent sneaky matings from other males and ensure paternity (Lamb 1976).

The courtship behaviour of *F. auricularia* is complex with 16 distinct behaviours observed (Figure 1-2) (Walker & Fell 2001). Males initiate courtship with forcep waving towards the female followed by backward movement towards the female with his forceps directed towards her abdomen and forceps. The male performs antennal drumming of the female. If the male is initially accepted by the female, several forcep displays are next performed including splaying, bobbing and raising, followed by the stroking of her abdomen, head and pronotum. These behaviours are followed by the male enclosing his forceps around the female's abdomen, cervix, head, or forceps with lateral movement along the female with his enclosed forceps for relatively long periods (> 1 hr). The female may reject the male at any point of the courtship with behaviours such as abdominal twists, head nodding or forcep bobbing displayed. The male pursues the female if rejected, trying to reinitiate courtship. If the female is finally receptive the male backs toward the caudal end and twists his abdomen 180˚, whilst the female raises her abdomen, bringing their ventral parts together. The male then slides backwards to enable copulation (Tomkins & Simmons 1998; Walker & Fell 2001).

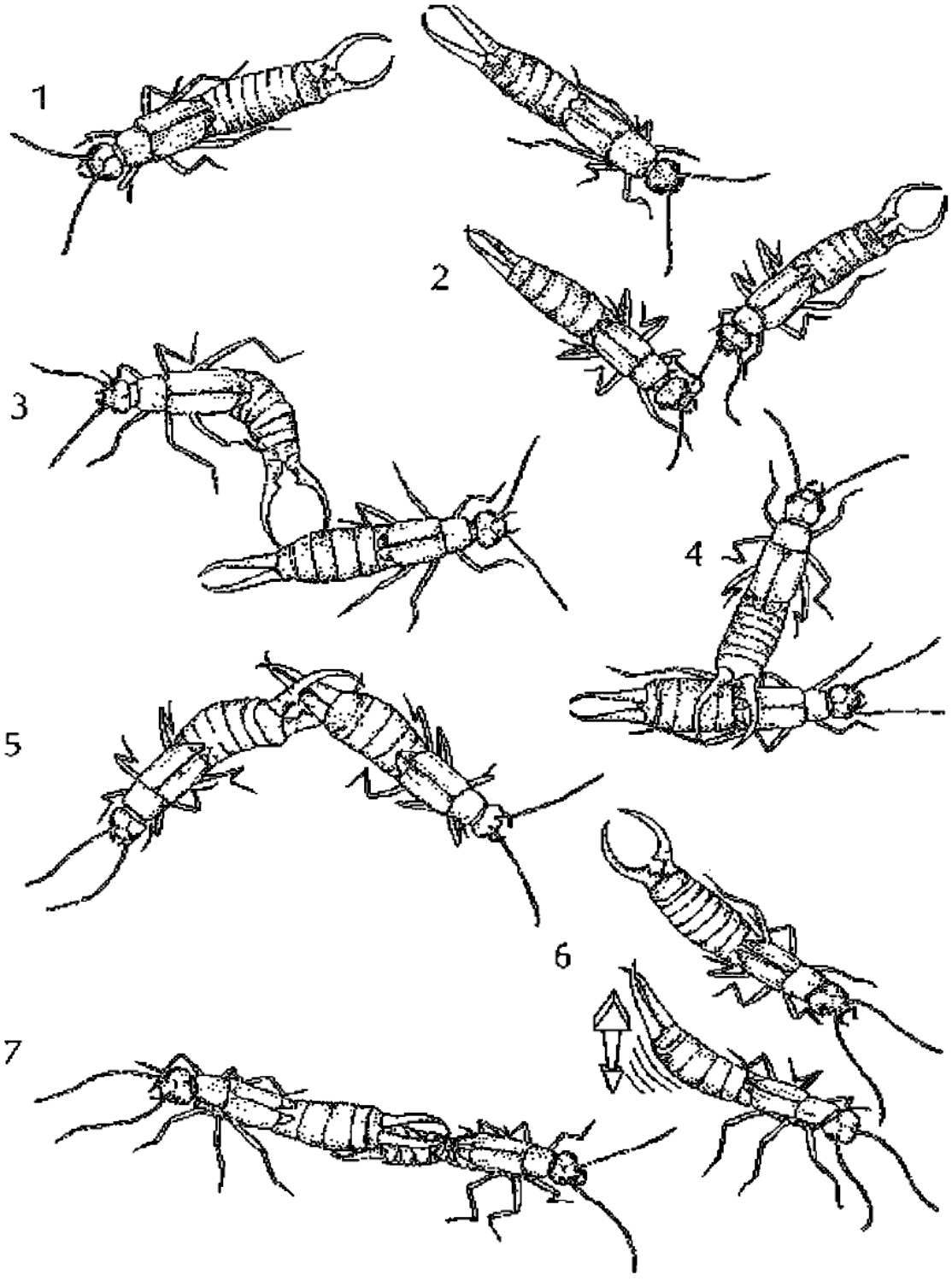

Figure 1-2. Courtship behaviour of *Forficula auricularia* (1) Male moves backward towards the female (2) Male antennates the female (3) Male displays forceps to female (4) Male strokes female with forceps (5) Male encircles female with forceps (6) Female raises abdomen (7) Copulation occurs (Walker & Fell 2001).

Egg laying occurs mid to late winter, with males then aggressively evicted from the nest by the females soon after oviposition, after which time the males soon die (Lamb 1976; Lamb & Wellington 1975). Eggs are 2 mm long, ovoid with a thin semi-transparent creamy-white to yellow chorion and are laid in single clutches of approximately 30-55 eggs and hatch in spring (Crumb *et al.* 1941; Helsen *et al.* 1998; Lamb & Wellington 1975).

Female earwigs show strong maternal care for both eggs and young nymphs with eggs turned and cleaned post-oviposition to limit fungal infection (Kolliker & Vancassel 2007). Brooding females provide food throughout the first nymphal instar via two behavioural mechanisms either food regurgitation or by direct provisioning i.e. whole aphids (Staerkle & Kolliker 2008). The frequency and longevity of the food provisioning phase is linked to the juvenile's cuticular hydrocarbon (HC) profiles, which fluctuate depending on the food resources (Mas *et al.* 2009). First instar nymphs remain in the nest with the female until the end of the first moult, when both nymphs and females leave the nest to either nocturnally forage in trees and leaf litter, returning to the nest by day or leave the nest permanently (Lamb & Wellington 1975).

After the juveniles leave the nest, subspecies B females then leave the nest and form another nest and lay their second, smaller clutch (Lamb & Wellington 1975; Wirth *et al.* 1998). Subspecies A females (one or two clutches per year) decision to lay a second clutch and the timing of the second clutch appear to be linked to a combination of cues particularly the timing of the first clutch, the juvenile cuticular HC quality signals and food availability (Mas & Koelliker 2011).

Helsen *et al*. (1998) estimated between 600-750 day degrees are required from oviposition to the final nymphal moult with a lower developmental threshold between 6-7 ˚C. Through summer and early autumn, adults are predominantly arboreal (Moerkens *et al.* 2009) feeding on vegetation and other insects (Bower 1992; Buxton & Madge 1977; Nicholas *et al.* 2005). During this free foraging phase, earwigs form mixed aggregations that contain both adult sexes and all life stages (Hehar 2007; Sauphanor 1992; Walker *et al.* 1993). Soon after the final moult a rapid decline in earwig populations has been observed in apple and pear orchards. The reasons for this decline currently are unclear as no evidence of dispersal, reduced food availability, increased natural enemy populations, disease or use of insecticides is evident (Moerkens *et al.* 2009; Quarrell 2008).

Forficula auricularia's aggregation pheromone

The production and regulation mechanisms of insect aggregation pheromones are as diverse as the morphology and life cycles of the insects that utilise them. Little is currently known about the aggregation pheromone utilised by *F. auricularia* (Sauphanor 1992; Walker *et al.* 1993). Sauphanor (1992) concluded the pheromone originated from the tibial glands, which Walker *et al*. (1993) later found to have a repellent effect. Walker *et al.* (1993) went on to demonstrate in laboratory-based bioassays that both male cuticular washes and frass from all members of the population contained the aggregation pheromone. They concluded that the pheromone originates from the male cuticle, which is later consumed post-ecdysis by other members of the population, and can be thereby found in the frass of the entire population (Walker *et al.* 1993). Unfortunately, the frass samples they analysed were not collected and isolated from the differing sexes and life stages and therefore their conclusions remain confounded.

Several hydrocarbons and both saturated and unsaturated fatty acids (FAs) ranging from C_{14} to C_{18} were identified from frass and cuticular washes of *F. auricularia* by Walker *et al*. (1993). Olfactometer based choice tests of these compounds yielded little success with only stearic and palmitic acids displaying attractancy at high concentrations (50 male equivalents/day) (Walker *et al.* 1993).

Hehar (2007) attempted to isolate *F. auricularia's* aggregation pheromone from several point sources, including the earwig's frass, abdominal defensive glands and the integument. Unfortunately, tibial gland extracts were not analysed, which were implicated by Sauphanor (1992) as being point sources of the aggregation pheromone. Hehar (2007) suggested that aggregation was not mediated by frass, but rather the compounds involved are volatile over short distances, of cuticular origin and produced and responded to by all members of the population. Analysis of headspace volatiles during this study isolated several never previously identified compounds including fatty acids, aldehydes, ketones, vanillin, numerous benzoquinones and an acetal, which were subsequently behaviourally tested as a number of differing blends. However, these blends only solicited responses in juveniles when the quinone fractions were removed with no single blend attracting all members of the

population as was demonstrated utilising substrates that were pre-exposed to earwigs in bioassays in the same study.

The attraction to these synthetic blends could be attributed to their use of hexadecanoic acid, which is known to occur in most living organisms (Wong & Koelliker 2012). This compound may have attracted these omnivores in a food-based response rather than an aggregative behaviour. Similarly, quinones are well known defensive compounds in earwigs and therefore may have had a repellent effect similar to that of an alarm pheromone. Flight responses are a commonly observed behaviour when earwigs emit benzoquinones (Walker *et al.* 1993) and therefore may have led to the lack of juvenile attraction observed when these compounds were included in the blends.

Like the experiments conducted by Walker *et al.* (1993) and Sauphanor (1992), the volatile samples collected by Hehar (2007) were isolated from groups of earwigs in the laboratory fed unnatural diets and housed in high densities. Several studies have previously shown that pheromone production in insects is reliant on the intake of the pheromonal precursors via dietary consumption or diffusion (via spiracles), or by hormonal regulation triggered by physiological or environmental triggers (Moore *et al.* 1995; Vanderwel 1994) such as short term manipulation of an insect's carbon source, water availability (Mas *et al.* 2009; Mavraganis *et al.* 2008) or interaction with conspecifics (Barth 1965; Dukas & Mooers 2003; Moore *et al.* 1995; Schal *et al.* 2003). Genetic factors may also have impacted of the outcomes of these studies as *F. auricularia* populations are known to contain two subspecies (Wirth *et al.* 1998). As earwig speciation has never been considered during previous earwig hydrocarbon (HC) chemistry studies, it is possible that differences do exist between subspecies which may explain the outcomes of previous research in this area.

One notable omission from the above mentioned aggregation pheromone studies are the numerous alkenes and alkadienes partially identified from juvenile earwig cuticles by Liu (2005). Walker *et al*. (1993) briefly mentioned the presence of a pentacosadiene ($C_{25:2}$) and heptacosadiene ($C_{27:2}$) but did not attempt to identify the double-bond positions of these compounds or their subsequent behavioural importance. Recently this suite of cuticular hydrocarbons were shown to be used in the maternal care behaviours of *F. auricularia* to mediate food provisioning to juveniles (Geiselhardt *et al.* 2009) and therefore may also play a role in other earwig behaviours including aggregation. However, these compounds have yet

to be characterised fully with several double-bond positions and methyl-branching points yet to be determined.

Use of pheromones to control earwigs

Pest and disease management remains a key issue in the maintenance of agricultural profitability and environmental health (Thomson & Hoffmann 2006). Prior to the beginning of the pesticide revolution that followed World War II the use of biological control agents was commonplace. Following World War II both researchers and producers came to recognise that there was no single "magic bullet", which would eliminate pest species. This led to the recognition that the presence of some pest species within a crop is inevitable and that pest minimisation was the target not pest elimination. In order to achieve this goal an integrated approach was developed that included biological, cultural, physical and mechanical control measures (Hagler 2000; Stern *et al.* 1959). Unfortunately, the utilisation of integrated pest management (IPM) practices has been slow with broad-spectrum insecticides still being commonplace (Brewer & Goodell 2012; Kaine & Bewsell 2008; Zalucki *et al.* 2009). This lack of IPM uptake has been attributed to numerous factors including few financial incentives, a lack of adequate education and extension services to aid producers in the development of IPM programs and a zero tolerance for pests within exported commodities (Kaine & Bewsell 2008). This continued reliance on chemically based pest management strategies subsequently led to continued issues with environmental pollution events, insecticide resistance and secondary pest outbreaks in many pest species (Gilliom 2007; Pimentel *et al.* 1992).

Due to insecticide resistance and environmental issues, the pendulum may be slowly swinging back towards the integrated approach. The use of modern scientific methods has provided producers and researchers with a swathe of new weapons in the pest control arsenal including the use of pheromones and a resurgence of interest in natural enemies (Khan *et al.* 2008). The use of insect semiochemicals has also become commonplace in many agricultural systems. Their applications vary with monitoring, mating disruption and lure and kill strategies all playing valuable roles in the control of many economically important insect pests (Khan *et al.* 2008; Suckling 2000).

Until recently the use of pheromones in agricultural insect control has mainly focused on sex pheromones. However, aggregation pheromones are now being used to boost natural enemy populations of the spined soldier bug, *Podisus maculiventris*, in home gardens, as a "lure and kill" control option or to enhance pest monitoring for Colorado potato beetle, *Leptinotarsa decemlineata* (Khan *et al.* 2008) and numerous weevil species (Ambrogi & Zarbin 2008).

As *F. auricularia* prefers temperate climates, with an annual rainfall >500 mm and winter temperatures < 24˚ C (Mueller *et al.* 1988), this species could be an ideal biological control agent for Tasmanian apple producers in the control of many pest species. If the aggregation pheromone of the European earwig is isolated, *F. auricularia* populations could possibly be manipulated in horticultural situations where they are deemed either a beneficial or pest species (Suckling *et al.* 2006). This pheromone could be used in conjunction with current Integrated Pest Management (IPM) strategies for either pest monitoring, trapping or used as a "lure and kill" control option. This would effectively increase the sustainability of orchard management practices and reduce the environmental impacts of growing both pome and stone fruit crops within Tasmania by reducing the use of broad-spectrum insecticides.

Earwigs as a biological control agent in apple orchards

European earwigs have long been regarded as an useful biological control agent against numerous insect pests in apple and pear orchards in particular soft bodied insects such as codling moth (*Cydia pomonella*) (Glen 1975) and woolly apple aphid (*Eriosoma lanigerum*, WAA) (Lea 1904; Nicholas *et al.* 2005; Suckling *et al.* 2006).

WAA, an aphid species endemic to North America, was first discovered in Australia in 1895 (Waterhouse & Sands 2001). WAA overwinters on branches and root systems forming hypertrophic galls on American elm (*Ulmus americana*) and apple trees (*Malus domestica*). Asexual reproduction and nymphal development continues whilst overwintering on roots with the aphids on branches remaining dormant until spring (Mols & Boers 2001). Root dwelling aphids emerge when soil temperatures reach approximately 10 ˚C and start colony development on the vulnerable or thinly barked aerial parts of the tree such as fresh growth, pruning cuts or broken branches and limbs. Once feeding has commenced the aphids remain largely sessile unless disturbed (Asante 1994). The development time for WAA ranges between 11.7 and 57.8 days at temperatures between 10 – 30 ˚C with lower and upper

development thresholds of 5.2 ˚C and 32 ˚C respectively (Asante *et al.* 1991). This rapid development time results in WAA being capable of up to 12 generations per year, reaching peak population size from February to March in the Southern Hemisphere (Asante 1994; Mueller *et al.* 1988). Although WAA do not directly damage the fruit they are capable of reducing yields and fruit quality and are also deemed a nuisance to fruit pickers due the waxy secretions they produce (Waterhouse & Sands 2001).

Three methods of WAA control are utilised in apple orchards: (1) aphid resistant rootstocks (Sandanayaka *et al.* 2003); (2) insecticides (Nicholas *et al.* 2003, 2005); and (3) augmentative (Carroll & Hoyt 1984) and conservation biological control (Suckling *et al.* 2006). Several WAA resistant rootstocks are available to apple producers. These provide a valuable method of reducing subterranean aphid populations, which are out of reach of predators and parasitoids during the aphid's overwintering phase. These rootstocks MM109 and M793 were originally derived from Northern Spy apple cultivars, which carry WAA resistance genes *Er1*, *Er2* and *Er3*. However, recent studies have shown that some aphid populations have developed resistance to *Er1* (Sandanayaka *et al.* 2003).

Biological control agents have long been recognised as viable controls for WAA populations in apple orchards worldwide (Mueller *et al.* 1988). Several taxa have been demonstrated to control aphid populations including parasitoids and predators such as Hymenoptera, Neuroptera, Coccinellidae and Dermaptera (Madsen & Morgan 1970). The parasitoid wasp *Aphelinus mali* has long been deemed the primary biological control agent used to manage WAA infestations (Nicholas *et al.* 2005). *A. mali* was first released in Australia from North America via New Zealand populations in 1923. It has provided excellent control particularly in warmer apple growing regions across Australia. The adult wasp lays in all nymphal instars and the adults of *E. lanigerum* with unfertilised eggs developing into male wasps (Mols & Boers 2001). Unfortunately, *A. mali*'s lower development threshold of 8.3 ˚C lags behind its aphid host (5.2 ˚C) (Asante *et al.* 1991), which culminates in the parasitoid only developing 4-5 generations per year compared up to 10-12 generations observed in WAA (Asante 1994; Mols & Boers 2001). This creates the potential to allow WAA populations to reach levels where fruit bud formation and extension growth are deleteriously affected before parasitoid numbers have a significant effect on WAA populations, particularly in temperate climates such as those in Tasmania.

As previously stated *F. auricularia* is an omnivorous insect with a preference for insect eggs and soft bodied insects including aphids and Lepidopteron larvae (Buxton & Madge 1977). This observation has led to earwigs being identified as a viable biological control agent in apple orchards against aphid pests including the WAA (Nicholas *et al.* 2005). The use of earwigs as biological control agents is not unknown including the use of Australian native species. *Elaunon bipartitus* and *Labidura truncata* are known to predate upon pink sugarcane mealy bugs (*Saccharicoccus sacchari*) and *L. truncata* and *Nala lividipes* eat bush fly larvae (*Musca vetustissima*), cabbage white butterfly larvae (*Pieris rapae*) and *Helicoverpa armigera* and *Heliocoverpa punctigera* larvae (Waterhouse & Sands 2001).

Conflicting reports exist with respect to the efficacy of *F. auricularia* as a biological control agent of WAA (Carroll *et al*. 1985; Nicholas *et al*. 2005). Asante (1995) showed in laboratory experiments that adult *F. auricularia* may attack up to 106 nymphs per day with consumption decreasing proportionally with increasing aphid size. Due to these potentially high rates of WAA consumption both natural and augmented earwig populations have been shown to significantly reduce aphid populations in apple orchards (Carroll & Hoyt 1984; Mueller *et al.* 1988; Nicholas *et al.* 2005). However, similar trials have shown the efficacy of earwigs as control agents can vary from season to season with adequate WAA control observed in apple trees in one year but not in the following year (Carroll *et al.* 1985). This could possibly be due to varying tree sizes (Carroll *et al.* 1985) or abundant food resources including alternative prey species in larger tree canopies (Asante 1995). Despite this variability in control, earwigs are more effective predators than other biological control agents such as ladybirds, lacewings and hoverflies in apple orchards (Nicholas *et al.* 2005).

Differing studies have produced various earwig population estimates per tree to adequately control aphid infestations. Nicholas *et al.* (2005) recommended between 4.98 and 8.30 earwigs are required per monitoring trap dependant on the apple cultivar, whereas Mueller *et al.* (1988) recommended numbers between 3.7 and 7.3 per refuge. These variations in earwig number may be due to the variety of monitoring methods utilised including trap design, trap placement, variations in tree size, ground cover management, the availability of alternative food sources, earwig sub-speciation and the timing of population estimates relative to earwig population dynamics. One clear need is to study the effectiveness of both *A. mali* and European earwigs together in acting on WAA population control.

In sweet cherries (*Prunus avium* L.), earwigs are regarded as a pest reportedly damaging fruit and are a potential issue in post-harvest packing, export and biosecurity (Bower 1992). In some stonefruits, such as apricots, European earwigs have been reported to damage up to 40% of some harvests (McLaren 1999). However, similar work into the impact *F. auricularia* has on cherry production is currently unknown, although in extension literature damage attributed to earwigs includes cherry leaf, fruit bud, pedicel and fruit damage in Australia (Bower 1992; Domeney & Williams 2002) and in the U.S.A. (Grant *et al.* 2005). This literature states earwig feeding results in shallow, irregular holes in the cherry fruits, which may also become infected with secondary fungal infections (Grant *et al.* 2006).

Despite its assumed pest status there has been no empirical research undertaken quantifying the impact earwigs have on cherry production or any action thresholds developed to determine insecticide usage in cherries. A web-search of university and governmental agricultural extension services found numerous documents stating that *F. auricularia* is a pest in cherries and provides chemical management strategies for their control (Antonelli 2006; Bower 1992; Domeney 2009; Grant *et al.* 2006; James 2011). It is therefore essential that any impact that earwigs may have on cherry production be quantified to determine whether these anecdotal reports are accurate, particularly as broad-spectrum insecticide applications remain the primary method of earwig control.

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Chapter 2 Mapping of the subspecies complex of the invasive earwig, *Forficula auricularia* in Australasian ecosystems

Formatted for the journal "Biological Invasions"

Abstract

The European earwig, *Forficula auricularia*, is a cosmopolitan insect endemic to Europe, west Asia and possibly North Africa, which has invaded many temperate regions of the world including Australia and New Zealand. Recently, *F. auricularia* has been shown to be a complex of two subspecies, which display differing reproductive strategies. If the subspeciation and invasion distribution of populations are known, earwig management strategies could be better targeted in agricultural and urban environments. To develop a greater understanding of the invasion biology of *F. auricularia* in Australia we first examined Australian *F. auricularia* entomological collections and historical literature and made further field collections to determine its Australian distribution. We then undertook a genetic analysis of *F. auricularia* collected from Australia and New Zealand using two mitochondrial genes (COI and the COI-COII intergenic region). These were compared to sequences from 17 locations within its European range to provide insights into its invasion biology and identify possible source populations within Europe. The historical records examined indicate that *F. auricularia* was first introduced onto the island of Tasmania as early as 1847; with the Australian mainland introduction occurring by 1900. Its present distribution is localised to disturbed ecosystems within the temperate regions of southern Australia. Genetic analysis indicated that Australian and New Zealand populations are comprised solely of subspecies B. Within this subspecies, Tasmanian and New Zealand populations consist of a single clade comprised of 4 and 1 haplotypes respectively, whereas Australian mainland populations also contain a second clade and up to 11 haplotypes indicating that multiple introductions probably occurred on the Australian mainland. Comparison of mitochondrial genomes from Australasia and European populations revealed that one clade was widely dispersed throughout Europe but the other clade was not identified within our European sampling range. Continued sampling efforts across its endemic distribution, coupled with microsatellite analysis would help determine the sources of the Australian and New Zealand introductions and the size of the original invasive populations.

Keywords: *Forficula auricularia*, Australia, biological invasion, earwig, population bottleneck

Introduction

The tendency of new world settlers to acclimatise recently established areas with the deliberate importation of plants and animals from their countries of origin has increased the likelihood of accidental introductions of both pestiferous and innocuous species across the world (Cassey et al. 2004). This movement of invasive species has been further exacerbated by the development of modern domestic and intercontinental transport systems (Liebhold and Tobin 2008). However, the success of an introduction is dependent on numerous favourable biotic and abiotic factors including the absence of natural enemies, propagule pressure, the organisms dispersal ability (Liebhold and Tobin 2008) and capacity to exploit its new environment including food resources (Snyder and Evans 2006; Cassey et al. 2004). Although not all incursions overcome these factors and become established many have, and have gone on to become urban and agricultural pests or cause serious issues to human and ecosystem health (Lach and Thomas 2008).

The European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), is one such insect that has managed to overcome the issues faced by an introduced species on numerous occasions. Native to Europe, western Asia and possibly northern Africa (Lamb and Wellington 1975), *F. auricularia* are now established in most temperate regions of the world including Australia, New Zealand, North America and South America (Rentz and Kevan 1991). In many ecosystems this insect is regarded as an agricultural (Suckling et al. 2006; Gordon et al. 1997; Kehrli et al. 2012) and urban pest (Lamb and Wellington 1975; Walker et al. 1993).

Since its invasion into Australia, *F. auricularia* has become common throughout south-eastern Australia. It was first scientifically documented in Australia by Tasmania's first state entomologist in 1903 (Lea 1903). However, it may have been introduced into Tasmania much earlier. European earwigs were first scientifically reported in mainland Australia within an apple orchard located outside Sydney in 1930 (Gurney 1934), after which point they appear to have spread rapidly. In 1994, the first record of this species was made outside Albany, Western Australia, which is geographically isolated by the Nullarbor Plain from Australia's east coast (Widmer *et al.* 2008). Since this time *F. auricularia* has continued to spread through south-western Western Australia reaching Perth in 2011 (Widmer, M 2011, pers. comm., 4th March). The timing of *F. auricularia*'s introduction into New Zealand is

unknown, however, attempts were already being made by 1924 to introduce the parasitoid flies; *Digonochaeta setipennis* (Fallén) and *Rhacodineura antique* (Meig) to curb its' spread (Fulton 1924) indicating it had already reached problematic numbers.

The impact that *F. auricularia* is currently having on Australia's endemic fauna has yet to be examined. However, *F. auricularia* has been implicated in the decline of several threatened and endangered invertebrate species in America, including the Valley Elderberry Longhorn Beetle (*Desmocerus californicus dimorphus* Fisher) (BDCP 2008) and the El Segundo Blue Butterfly (*Euphilotes bernardino allyni* Shields) (Mattoni 1998). Therefore, endangered ground dwelling Australian invertebrates such as the Golden Sun Moth, *Synemon plana* (Walker) (O'Dwyer *et al.* 2004) or the Ptunarra Brown Butterfly, *Oreixenica ptunarra* (Couchman) (Bell 1998) may also be under increased threat from predation by *F. auricularia*.

Previously, climate and locality were solely believed to affect *F. auricularia*'s life-history. However, genetic analysis of populations in Europe and North America identified two subspecies with differing reproductive strategies, subspecies A (one or two clutches per year) residing in the Pyrenean alpine zone >1100 m, and subspecies B (two clutches per year) residing between sea level and 1200 m (Wirth et al. 1998; Guillet et al. 2000b). Analysis of a 623 bp mtDNA fragment identified interspecific genetic divergence five to seven times greater than the intraspecific variation. Studies have shown that where these subspecies coexist no sexual interaction is apparent due to the lack of hybridised individuals (Wirth et al. 1998). Furthermore, forced copulations between subspecies in laboratory experiments showed egg infertility to be prohibiting any genetic flow between populations (Wirth et al. 1998; Guillet et al. 2000a). The genetics of Australian *F. auricularia* populations is currently unknown but if determined could aid population monitoring in agricultural and natural resource management scenarios by enabling more precise prediction of earwig population dynamics, adaptation and resilience.

In this study we utilised all available Australian *F. auricularia* entomological collection information, historical literature and our own field collection data to determine the Australian distribution of *F. auricularia* and possible points of entry into Australia. Genetic analysis of *F. auricularia* mitochondrial genomes from both within its endemic European range and Australian and New Zealand populations was also undertaken to provide further insights into its invasion biology and possible source populations within Europe.

Materials and methods

Invasion timeline estimates

To determine the time of *F. auricularia*'s introduction into Australia archival scientific and historical reports were examined via the National Library of Australia's Trove database (http://trove.nla.gov.au) using "earwig", "Dermaptera" and "Forficula" as key word searches. To ensure false reporting did not occur the context in which each report mentioned any of the search terms was scrutinised thoroughly with reports of the common, garden, pest or introduced earwig deemed to be accurate. Australian native earwigs are not generally regarded as pests (Rentz and Kevan 1991), therefore it is highly likely that these reports are discussing *F. auricularia*.

Australian distribution mapping

To determine *F. auricularia*'s Australian distribution, collection records were compiled from entomological collections held by all state government Department of Primary Industries, state museums and the CSIRO's Australian National Insect Collection (ANIC) and supplemented with our own field collection data. All records were then mapped using the species mapping and analysis function on the Atlas of Living Australia website (http://www.ala.org.au/).

Genetic analysis

Sample collection in Australasia

We collected samples from 28 sites (n = 612 individuals) around Australia on three field trips (Table 2-1) and from two locations (n = 15 individuals) in New Zealand (Rotorua: 38° 07.002' S, 176° 19.002' E, Diamond Harbour: 43° 37.000' S, 172° 43.999' E). Dedicated effort was placed on determining *F. auricularia*'s northern boundaries and collecting samples from higher elevations. Samples were collected as adults whenever possible in order to most easily confirm identification. A maximum of 2 days was spent searching for specimens at all locations including those where specimens could not be found (Table 2-1). The sampling area at each site was ca. 100 m^2 with a maximum of 2 individuals collected from any single earwig aggregation to prevent the subsequent analysis of individuals from within the same family group.

DNA isolation, amplification and restriction

To determine Australian and New Zealand population sources their genetics were compared with *F. auricularia* sequences (n = 158) from both subspecies, collected from 17 locations across Europe including France, Germany, Belgium, Denmark, Switzerland, Italy, Turkey and the United Kingdom obtained by Prof. Thierry Wirth (Muséum National d'Histoire Naturelle, Paris, France). Genetic analysis was undertaken by Prof. Thierry Wirth. Total DNA was extracted from the head and thorax of 162 individuals collected in Australia and 11 samples from New Zealand individuals using a standard CTAB extraction protocol. Two mitochondrial regions were then amplified by PCR; a portion of COI (658 bp) and the COI - COII intergenic region (497 bp) using restriction enzymes Bsr 1 and Afl II and subsequently sequenced.

Genetic data analysis

Population genetic analyses

Pairwise nucleotide diversity (π) and the number of segregating sites (Watterson's theta, θ_w) were calculated with DnaSP, version 4.10 (Rozas *et al.* 2003). Two tests to assess population expansion were also performed: Tajima's D (Tajima 1989), Fu's Fs (Fu and Li 1993), as well as Ka/Ks ratio test (Yang and Bielawski 2000).

Demographic inferences

To determine whether some mtDNA lineages underwent recent population expansions mismatch distributions were calculated and compared to predicted population expansion models (Rogers 1995). For expanding populations we converted the parameter Tau (τ) (calculated from the mismatch distribution) to estimate the time of expansion (t) using the equation $\tau = 2\mu t$, given that $\mu = 2\mu k$ and μ is the mutation rate per nucleotide and k is the sequence length (Rogers and Harpending 1992). The confidence intervals of τ were then calculated using a parametric bootstrap approach (Schneider and Excoffier 1999). Mismatch distributions were then calculated using ARLEQUIN 3.0 (Excoffier *et al.* 2005).

Phylogenetic inferences and coalescent analyses

Phylogenetic relationships were reconstructed using the neighbour-joining (NJ) algorithm implemented in MEGA 5.1. The robustness of the NJ tree topology was assessed

with bootstrapping analyses of 1,000 pseudo-replicated datasets. A generalized time reversible (GTR) substitution model with gamma distributed rate heterogeneity and a proportion of invariable sites were selected based on Akaike's information criterion (AIC) using JMODELTEST.

Population size changes through time and "Time to Most Recent Common Ancestor" (TMRCA) estimates of the main lineages were obtained using the Bayesian MCMC approach implemented in BEAST. The specific rate of evolution for the mtDNA fragments was fixed at 3.54×10^{-8} as reported by Papadopoulou *et al.* (2010). Evolutionary rates and tree topologies were analysed using the GTR and Hasegawa-Kishino Yano (HKY) substitution models with a gamma distribution and an among-site rate variation with four rate categories.

A Bayesian skyline model based on a general, non-parametric prior that enforces no particular demographic history was used to determine changes in *F. auricularia*'s effective population size through time (Ho and Shapiro 2011). For each analysis, a piecewise linear skyline model with 10 groups was used and two independent runs of 50 million steps performed. Examination of the MCMC samples using TRACER 1.4 indicated convergence and adequate mixing of the Markov chains, with estimated sample sizes in the hundreds or thousands. The first 10% of each chain were discarded as burn-in.
We summarized the MCMC samples using the maximum clade credibility topology found with TREEANNOTATOR, with branch lengths depicted in years (the median of those branches were present in at least 50% of the sampled trees). The Bayesian skyline plot was then reconstructed using the posterior tree sample with TRACER 1.4.

Results

Historical literature search

F. auricularia appears to have been introduced into Tasmania, Australia, as early as the late 1840's when newspaper reports discuss earwig control for Dahlia flowers within home gardens in Hobart (The Cornwall Chronicle 1847). However, they did not appear to reach problematic levels in the Hobart area until the late 1870's when several articles were published complaining of earwigs causing "the utter destruction of all vegetation" in home gardens (The Mercury 1878, 1879). Similarly, reports though limited, describe its spread

though Tasmania for example in 1884 a report discusses the condition of that seasons stonefruit crops on Tasmania's South Arm, 40 km south of Hobart stating that "the earwig has not reached this far" (The Mercury 1884) and a report in 1886 describing "the earwig which swarms in Hobart has made his way to New Norfolk" (The Argus 1886) with New Norfolk 35 km north-west from Hobart.

The first Australian mainland reports that mention earwigs appear in 1841 where a report in the "South Australian Registrar" states "we gladly look in vain for a single species". However, in 1888 an article in the "South Australian Register" mentions the donation of a "Farficula (earwig)" to the South Australian Museum by Angus H. McBride collected at Tantanoola, 410 km east of Adelaide (South Australian Register 1888). However, whether this reference is to *F. auricularia* is unclear. Soon after this report numerous articles in southern Australian newspapers specifically addressing the "introduced", "common" or "garden" earwig in NSW (Australian Town and Country Journal 1900; Liverpool Herald 1901), Victoria (West Gippsland Gazette 1907; The Argus 1911) and South Australia (White 1915; The Register 1912; The Mail 1918) were published. In Western Australia, during the course of this study an increasing number of *F. auricularia* populations were being discovered in the outer suburbs of Perth ca. 400 km from Albany where they were first discovered in 1994, indicating they are continuing to disperse across south-west Western Australia (Widmer, M, pers. comm., 4th March 2011).

Distribution mapping

Insect collection records from museum and governmental insect collections and our own field collections yielded 164 different locations around Australia where *F. auricularia* have been recorded (Figure 2-1). An additional female specimen collected in 1960 inside a building at the sub-Antarctic research station on Macquarie Island (1500 km south east of Tasmania) was also found within the Australian National Insect Collection (ANIC). During our field collections no *F. auricularia* were found at several sites particularly in sub-tropical or xeric environments (see Table 2-1). Similarly, no records of *F. auricularia* were found within the tropical areas of Australia. The most northern populations were found at ca. 30° of latitude at elevations greater than 730 m above sea level at Armidale (S. Quarrell, pers. obs.) with one specimen found from Ballina within the ANIC holdings (28° 49.999' S, 153° 31.998' E). Though apparently unable to inhabit xeric environments we found populations in some semi-arid regions including Hay, NSW which has an annual mean rainfall below 370 mm

(www.bom.gov.au). Mapping data indicate that *F. auricularia* appear to be localised to disturbed environments within temperate and semi-arid areas of Australia (Figure 2-1).

Table 2-1. *Forficula auricularia* collection site data, subspeciation and clades (B_1 or B_2) determined using COI and the COI-COII intergenic regions (see Figures 2-2, 2-3 and 2-5).

Location	Site ID	Latitude	Longitude	Elevation (m)	Subspecies	COI	COI-II
Cooma	NSW2	-36º 13.917'	149º 07.303'	800	B	1 & 2	1 & 2
Adaminaby	NSW3	-35º 59.771'	148º 46.479'	1031	B	2	2
Batlow	NSW4	-35º 30.661'	148º 07.717'	866	B	1 & 2	1 & 2
Tumut	NSW5	-35º 15.530'	148º 14.738'	266	B	1 & 2	1 & 2
Young	NSW6	-34º 18.936'	148º 17.603'	436	B	1 & 2	1 & 2
Bathurst	NSW7	-33º 25.652'	149º 33.457'	711	B	2	1 & 2
Katoomba	NSW8	-33º 43.079'	150º 18.712'	995	B	1	1
Hay	NSW9	-34º 30.415'	144º 50.526'	100	B	2	2
Coffs Harbour	NSW10	-30° 17.776'	153° 06.811'	21		Not found	
Armidale	NSW11	-30º 32.640'	151º 37.200'	990	B	1	1 & 2
Dubbo	NSW12	-32° 14.577'	148° 36.291'	275		Not found	
Narrabri	NSW13	-30° 19.567'	149° 47.023'	240		Not found	
Tamworth	NSW14	-31° 05.429'	150° 55.742'	383		Not found	
Tanunda	SA1	-34º 31.649'	138º 57.919'	271	B	1 & 2	1
Coonawarra	SA2	-37º 17.494'	140º 50.064'	67	B	1 & 2	1
Jamestown	SA3	-33° 12.319'	138° 36.301'	440		Not found	
Geeveston	TAS1	-43º 08.260'	146º 54.460'	115	B	2	2
Bellerive	TAS2	-42° 52.633'	147° 22.400'	27	B	2	2
Westbury	TAS3	-43° 31.565'	146° 50.037'	167	B	2	2
Upper Natone	TAS4	-41° 13.516'	145° 54.616'	326	B	2	2
Bothwell	TAS5	-42º 22.991'	147º 00.517'	362	B	2	2
Geelong	VIC1	-38° 03.768'	144° 21.972'	17	B	1 & 2	1 & 2
Kingston	VIC2	-37° 22.152'	143° 56.343'	520	B	2	2
Sale	VIC3	-38° 06.592'	147° 04.250'	17	B	1	1
Bright	VIC4	-36° 43.614'	146° 57.745'	308	B	1 & 2	1 & 2
Shepparton	VIC5	-36° 23.785'	145° 23.791'	111	B	1 & 2	1 & 2
Mildura	VIC6	-34° 10.324'	142° 11.202'	48	B	1	1 & 2
Horsham	VIC7	-36° 42.557'	142° 11.533'	134	B	1 & 2	1 & 2
Hamilton	VIC8	-37° 44.451'	142° 01.850'	183	B	1 & 2	1 & 2
Ravensthorpe	WA1	-33º 34.897'	120º 02.886'	227	B	1	1
Pemberton	WA2	-34º 26.587'	116º 02.213'	123	B	1 & 2	1 & 2
Frankland	WA3	-34º 22.495'	117º 04.220'	230	B	1 & 2	1 & 2
Gairdner	WA4	-34° 07.560'	118° 43.720'	176	B	1	1

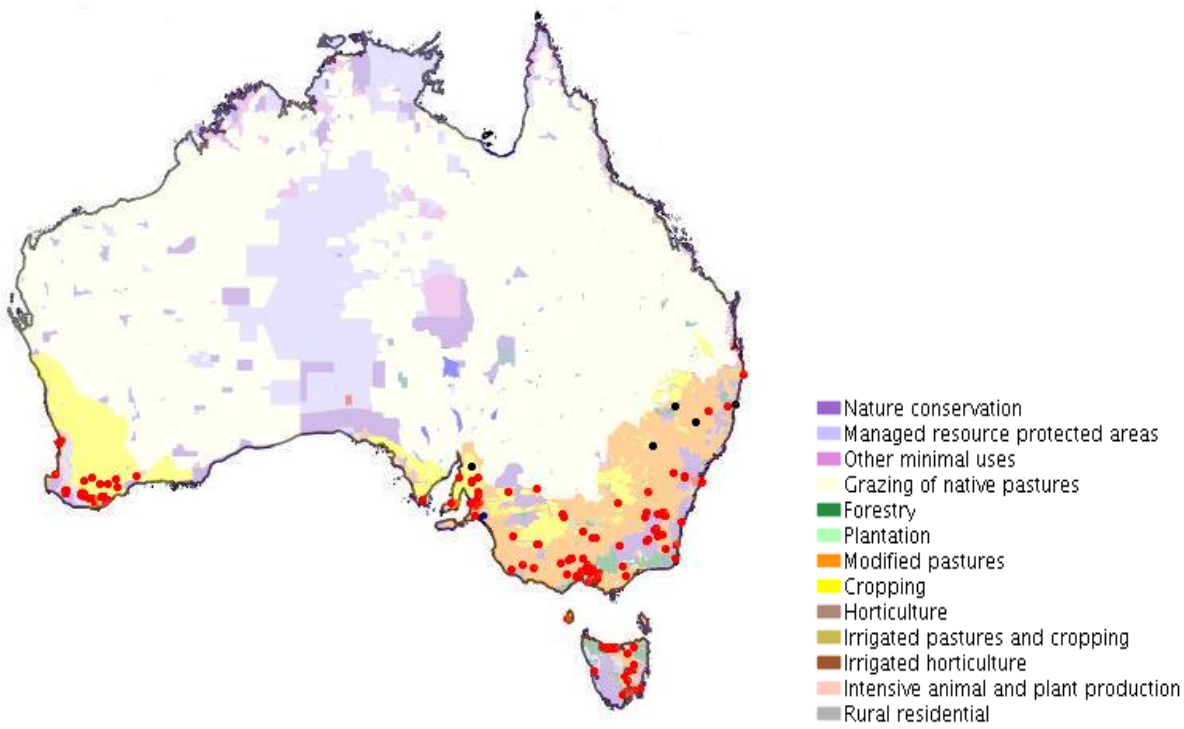

Figure 2-1. Australian distribution (indicated by red dots) of *Forficula auricularia* with land use overlay collected from entomological collections and field collection data. Black dots indicate sites where *Forficula auricularia* could not be located during field collections. Distribution map produced using the Atlas of Living Australia website.

Genetic analysis

Genetic analysis of Australian *F. auricularia* from 28 Australian sites and 2 sites in New Zealand show that all Australian and New Zealand populations consist of subspecies B (Table 2-1, Figures 2-2 and 2-3), meaning they produce two generations per year (Wirth et al. 1998). Both mitochondrial amplicon sequences within subspecies B show that two clades with reasonable haplotypic diversity exist within this subspecies, which we assigned as clades B_1 and B_2 (Table 2-2, Figures 2-2 and 2-3). These sequences also demonstrate that the subspecies A which was not discovered in Australasia, is also further divided into two separate clades with clade A_1 only isolated in Turkey and Greece thus far, and clade A_2 the dominant clade within European subspecies A populations, being present in at least Belgium, Switzerland, Italy and several locations in France.

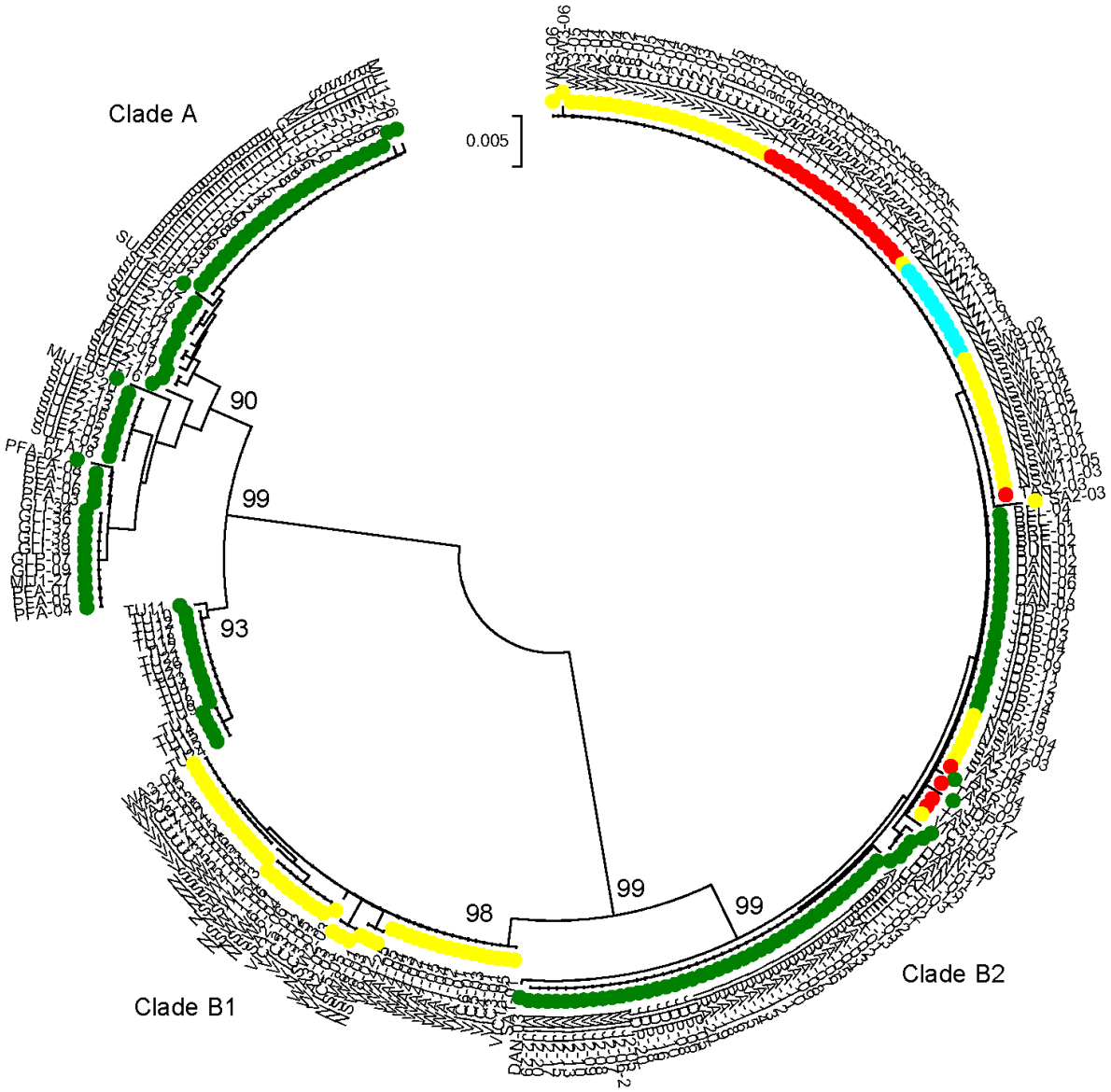

Figure 2-2. Cytochrome oxidase I (COI) neighbour-joining tree of 287 *Forficula auricularia* individuals collected from Australia, Europe and New Zealand. Genetic distances are based on the General time Based Model with gamma distribution and invariable sites. The bootstrap values are represented on the branches. The different colour codes correspond to differing geographical sources mainland Australia (Yellow), Tasmania (red), Europe (Green) and New Zealand (Light Blue). The differing haplotypes are distinguished by the differing branches within each clade.

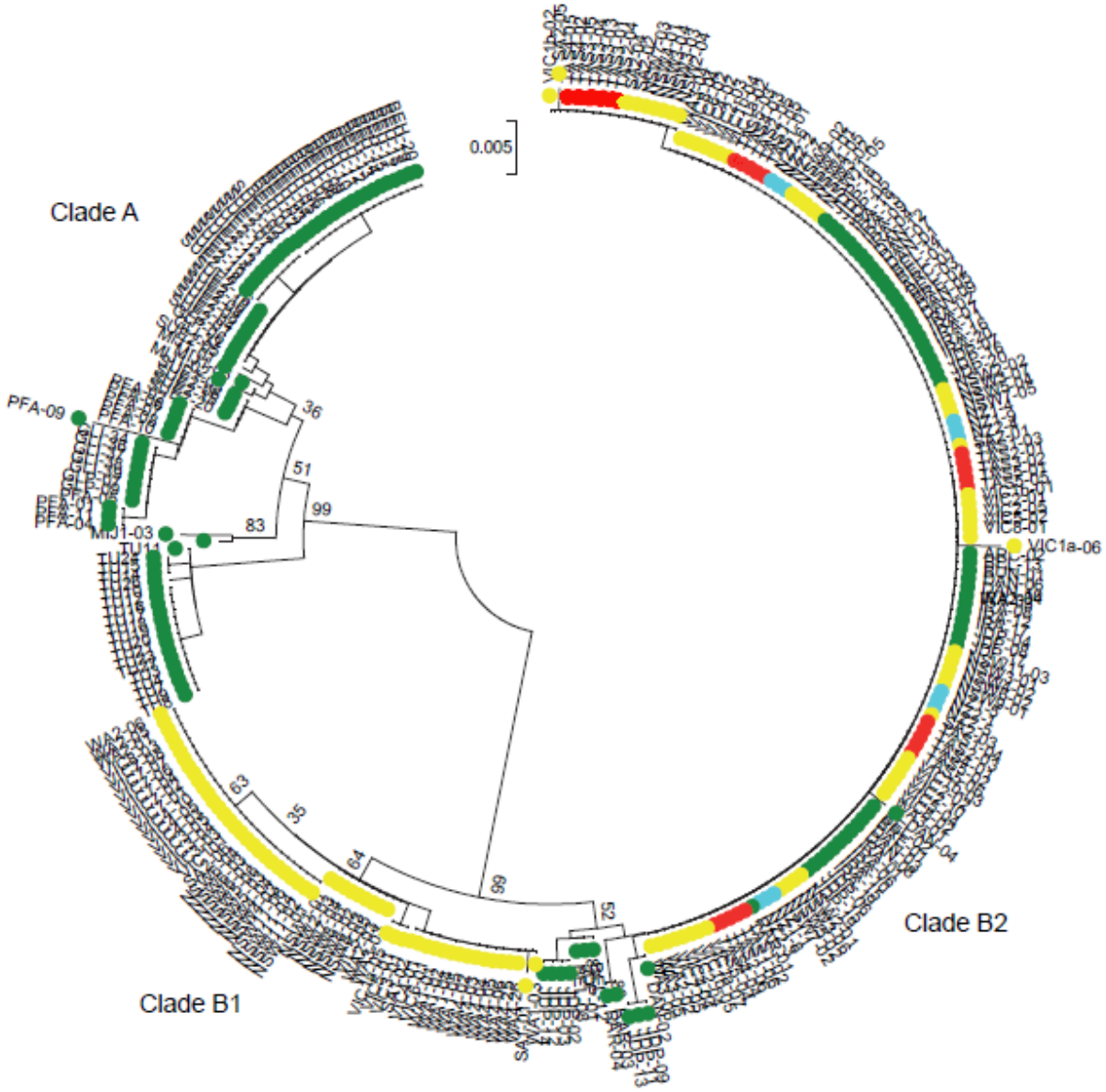

Figure 2-3. Cytochrome oxidase I-Cytochrome oxidase II (COI-COII) intergenic amplicon neighbour-joining tree of 300 *Forficula auricularia* individuals collected from Australia, Europe and New Zealand. Genetic distances are based on the General time Based Model with gamma distribution and invariable sites. The bootstrap values are represented on the branches. The different colour codes correspond to differing geographical sources mainland Australia (Yellow), Tasmania (red), Europe (Green) and New Zealand (Light Blue). The differing haplotypes are distinguished by the differing branches within each clade.

Despite numerous European populations being analysed, the origin of clade B_1 remains unresolved as no populations from this clade have yet been discovered within continental Europe or the United Kingdom. Despite the failure to locate B_1 populations within its endemic range, several B_2 populations were isolated in Europe including in Germany, Belgium, France and Denmark (Figures 2-2 and 2-3). Though unlikely due to the slow genetic mutation rates exhibited in many insects (3.54%/Myr) we investigated the likelihood of clade B_1 having evolved since its Australasian introduction using TMRCA calculations. The TMRCA data (Figure 2-4) indicates that the divergence between clades B_1 and B_2 occurred long before their introduction into the Australasian region, with subspecies B diverging and forming clades B_1 and B_2 ca. 67,000 years ago (95% HPD; clade B_1: 39,000 to 102,000; clade B_2: 45,000 to 91,000 years ago) indicating a more rigorous sampling effort is required to locate the source of these populations. Interestingly, these data also show that subspecies A and B diverged from its most common recent ancestor (MCRA) between ca. 142,000 to 145,000 years ago (95% HPD; subspecies A: 95,000, 200,000; subspecies B: 92,000 to 198,000 years ago) and that subspecies A populations found in Greece and Turkey may be the ancestral lineage with the more common clade A_2 diverging ca. 102,000 years ago (95% HPD; 65,000 to 141,000).

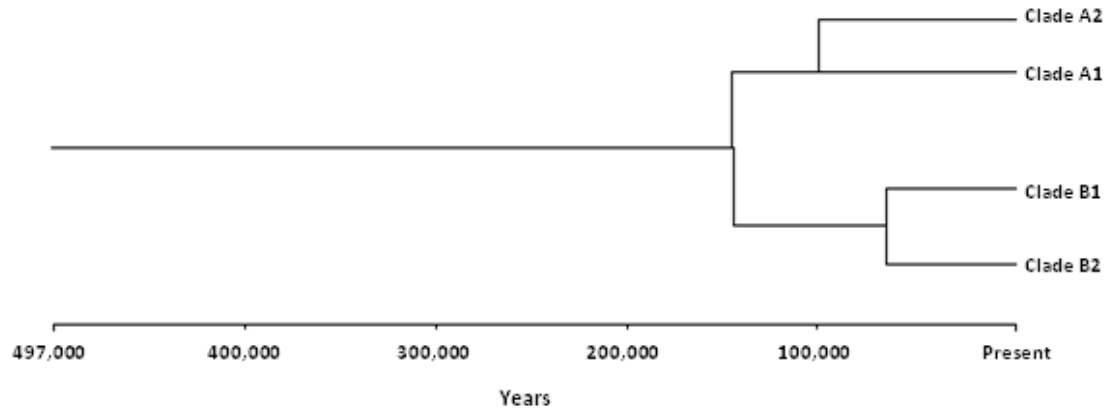

Figure 2-4. Representation of the genetic divergence of *Forficula auricularia* calculated using Bayesian estimates of the time to the most recent common ancestor (TMRCA) of the principle mitochondrial lineages under the Yule model implemented in the BEAST algorithm using a strict clock model ($\mu = 3.54 \times 10^{-8}$) from Papadopoulou et al. (2010).

Between these two subspecies (A and B) differences are evident with respect to the nucleotidic diversity (π) and mutation rate (θ_w) (Table 2-2), where subspecies A contains greater π and θ_w values. Within subspecies B, clade B_2 contains a greater number of

polymorphic sites and haplotypes (n = 11) but like clade B_1 still appears to have a relatively low genetic diversity (θw = 0.003). However, despite this low diversity within clade B_2, the significantly negative Fu's tests for both the COI and COI-COII intergenic fragments (COI: F_s = - 4.040, $P < 0.05$; COII-COII: F_s = - 1.772, $P < 0.05$) indicate that this lineage has gone through a recent genetic expansion, with the time since the expansion began calculated at ca. 23,696 years ago equating to the time around the last European glaciations (Clark et al. 2009). Similarly, a significant negative Tajima's D for the COI-COII fragment (D = - 1.866, $P < 0.05$) indicates an increase in population size after a bottleneck with any deleterious mutations occurring during this expansion being under negative selection pressure as indicated by the very low dN/dS ratios. Clade B_1, which has currently only been observed in Australia, appears to have also undergone a genetic bottleneck in the more recent past as both F_s and D values, though not significant, are either positive or near zero for both fragments (Table 2-2), which are indicative of a recent bottleneck with no recent expansion occurring. However, as endemic European populations of clade B_1 have yet to be found these values are more indicative of a small number of individuals introduced into Australia rather than characteristic of the clade.

Table 2-2. Population genetic analyses of the different *Forficula auricularia* mitochondrial lineages based on the cytochrome oxidase 1 gene (COI) and cytochrome oxidase I-cytochrome oxidase II intergenic region (COI-COII). * indicates significant difference at $P < 0.05$

Amplicon	Lineage	Length	n	S	h	π	θw	D	F_s	ω
COI	A	658 bp	84	39	18	0.011	0.012	- 0.213	- 1.372	0.03
	B_1	658 bp	47	8	7	0.002	0.003	- 0.465	0.303	0.04
	B_2	658 bp	155	11	11	0.003	0.003	- 1.244	- 4.040*	0.01
COI-COII	A	497 bp	83	30	18	0.013	0.012	- 0.237	- 0.805	0.03
	B_1	497 bp	57	4	4	0.002	0.002	0.481	0.068	0.04
	B_2	497 bp	160	12	10	0.001	0.004	- 1.866*	- 1.772*	0.01

n = sample size; S = number of polymorphic sites; h = number of haplotypes; π = nucleotidic diversity; θw = Waterson's Theta; F_s = Fu's F; D = Tajima's D; ω = dN/dS ratio

Of the populations of subspecies B found in Australasia, only clade B_2 was observed in Tasmania and New Zealand. Both clades, B_1 and B_2 were recorded on mainland Australia with higher proportions of clade B_1 evident around the Sydney region and throughout central Victoria (Figure 2-5). Within the B_1 clade, a total of 7 haplotypes occur within the COI fragment (amplicon) and a further 4 haplotypes in the COI-COII fragment (Table 2-2). The Australasian clade B_2 populations contain a total of 4 haplotypes within the COI fragment and 4 haplotypes within the COI-COII intergenic fragment. The number of haplotypes

observed in the Australian population appears far more diverse compared to that of New Zealand, Tasmania and Western Australia. Though based on a small sample size (only 2 sites) the two gene fragments sequenced show that only one haplotype exists in New Zealand, with this haplotype also found in Tasmania and on the Australian mainland (Figures 2-2 and 2-3). This low diversity indicates that few individuals from possibly a single family group were introduced into New Zealand with the progeny of these individuals sufficient to colonise both the North and South Islands despite a high level of inbreeding. Similarly, only two haplotypes were observed in Western Australia within the five locations sampled. The COI haplotype common to New Zealand and Australia has yet to be isolated in Europe and it is therefore unclear as to where this haplotype may have originated (Figure 2-2). The sequence data also provides evidence of mixing between the B_1 and B_2 clades in Australia as evidenced by the presence of both clades at some Australian sites (Table 2-1, Figure 2-5).

The possibility of a small invasion size in the Australasian region is further supported by both the low nucleotide diversity and number of haplotypes in the region compared to those in Europe (Table 2-3). When clade B_2 is examined by region, the number of polymorphic sites, haplotypes, the nucleotidic diversities and effective population sizes (as indicated by the differences in θ_w) in the Australasia populations are all approximately half that observed in Europe. This definitively points to a recent bottleneck followed by a population expansion in Australasia.

Table 2-3. Population genetic analyses of the B_2 *Forficula auricularia* mitochondrial lineage based on the COI and COI-COII intergenic fragments isolated from European and Oceanic populations.

Amplicon	Locality	n	S	h	π	θw
COI	Europe	74	9	9	0.00120 (0.00016)	0.00281
	Australasia	82	5	4	0.00051 (0.00014)	0.00153
COI-COII	Europe	64	8	7	0.00157 (0.00038)	0.00345
	Australasia	96	4	4	0.00070 (0.00014)	0.00159

n = sample size; S = number of polymorphic sites; h = number of haplotypes;
π = nucleotidic diversity; θw = Waterson's Theta

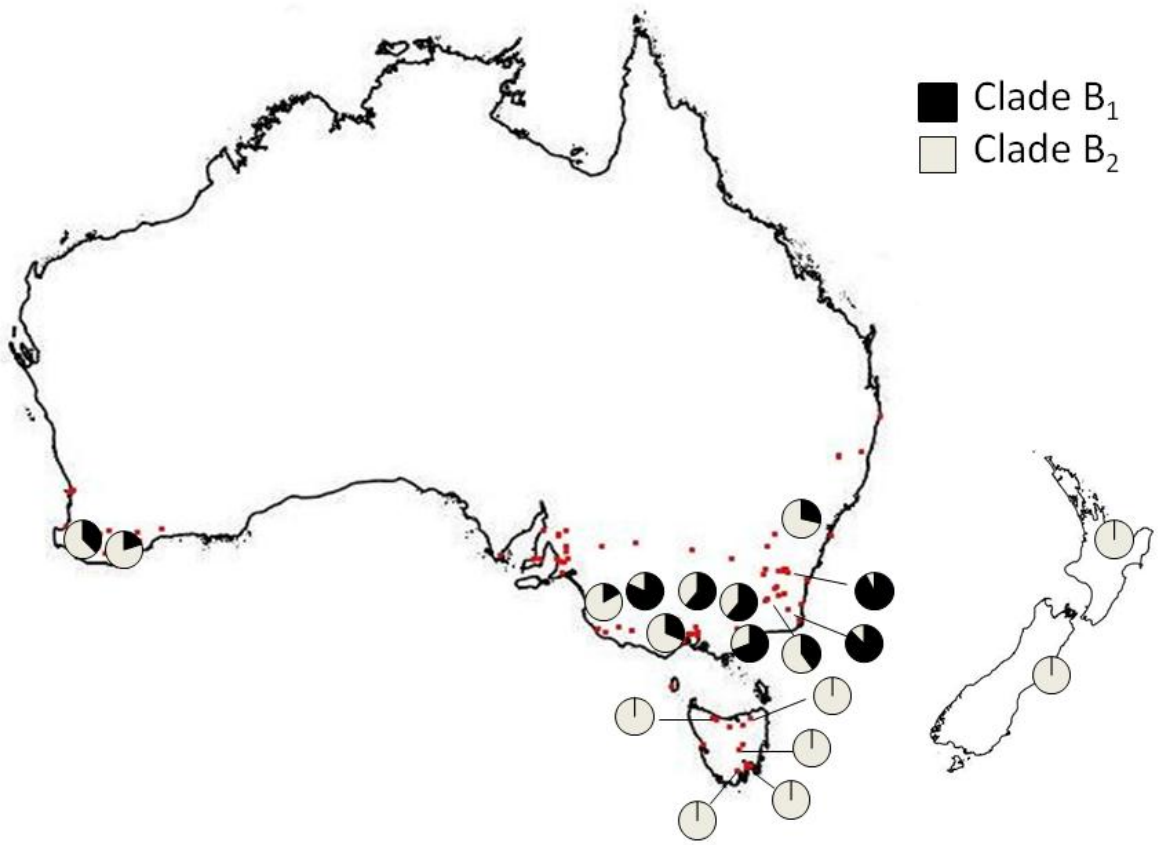

Figure 2-5. Distribution of Australian and New Zealand *Forficula auricularia* by clade. Red dots indicates site where *F. auricularia* have been recorded in Australia.

Discussion

Historical records show that *F. auricularia* may have been introduced into Australia as early as the late 1840's when Tasmanian newspaper reports begin discussing earwig control in home gardens in Hobart. They did not appear to reach problematic levels in the Hobart area until the late 1870's when several articles were published complaining of "the utter destruction of all vegetation" in home gardens. Despite the increased movement between Australia and Britain at this time, we are still to find to find evidence of any of the clade B haplotypes observed in Australia in the United Kingdom. Pin-pointing the source of the 1840 invasion is further complicated by the high level of trade between Tasmania and other Commonwealth colonies during this period (Matheson 2000). Historical records also indicate that *F .auricularia* were introduced onto the Australian mainland much earlier than the first scientific report in 1934 suggests, with numerous prior reports in the early 1900's in south-east Australia discussing the "introduced", "common" or garden earwig. Unfortunately, the level of information in these reports only provides limited evidence of the rate of spread of this species.

The European earwig's Australian distribution appears to be largely limited to temperate regions. No populations were discovered in the sub-tropical coastal and arid inland areas either side of the most northern highland populations despite extensive searches in four locations being conducted in these areas of northern New South Wales (Table 2-1). Indeed, the most northern established populations above 33 degrees of latitude were located in the highlands at above 730 m of elevation. Small earwig populations were also recorded in Hay, NSW, which is a semi-arid location indicating that this species is able to endure a wider variety of environments than those observed in Europe. Due to its ability to exist in a variety of climates it would appear that *F. auricularia* may continue to spread further north into the Western Australian grain growing areas and cause issues within agronomic crops north of Perth. Therefore, it would be prudent to conduct climatic modelling to determine *F. auricularia*'s potential Western Australian distribution. As *F. auricularia* has not been recorded in Australian native ecosystems (S. Quarrell, pers. obs.) it seems unlikely to pose a threat to endangered arthropods such as the Ptunarra Brown Butterfly, *O. ptunarra*, which is restricted to native grasslands (Bell 1998).

The genetic analysis of Australian *F. auricularia* indicates that all Australian and New Zealand population are of subspecies B. Furthermore, the early colonising Tasmanian *F. auricularia* population may have been one of the possible sources of the Australian mainland invasion, as Tasmania commonly exported produce and plant materials to the Australian mainland during the mid to late 1800's (Matheson 2000). An alternative explanation is that they were introduced onto the Australian mainland from the same source population in Europe. Indeed, as both clades of subspecies B are present on the Australian mainland including several haplotypes of clade B_2, which are not found in Tasmania it seems probable that multiple introductions may have occurred from the same European source, with clade B_1 derived from another point of origin. Similarly, the samples collected in New Zealand though from only 2 locations indicate they are the same B_2 haplotypes as those found in Tasmania and thus possibly derived from the same point of origin in Europe.

The rate of *F .auricularia*'s spread across Australia suggests a "stratified dispersal", where introductions were made ahead of the invasion front, which subsequently merge hastening the spread of the species within its new environment (Liebhold and Tobin 2008). Studies on the Argentine ant have shown that this mechanism enabled it to spread approximately three times

faster than by diffusive spread alone (Suarez et al. 2001). The cryptic nature and omnivorous feeding habit of *F. auricularia* makes this species perfectly suited to this mode of dispersal as it is easily transported within plant material and other cargo over long distances as observed by an individual earwig being found at the Macquarie Island sub-Antarctic research station in 1960. Indeed, it has been observed that dietary generalism, as in the case for *F. auricularia*, coupled with invasion size are extremely important factors in the invasion success of an introduced species (Cassey et al. 2005).

The spread of many invasive species is also linked to propagule pressure (the invasion size and number of invasions), where multiple introductions of numerous individuals tend to be most successful in establishing themselves within new locations (Lockwood et al. 2009). It appears that in Australia at least two introductions of multiple individuals occurred. However, the invading population in Western Australia appears to be derived from fewer individuals with a single introduction event possible, as the number of haplotypes across both clades B_1 and B_2 is restricted to only 4 haplotypes. The invasive potential of *F. auricularia* is also evident by its successful establishment into New Zealand with only 1 haplotype recorded from the two sites, situated ca. 890 km apart and located on separate islands. The successful introduction of a species from a single incursion has been observed in other species such as the bumble bee, *Bombus terrestris*, which appears to have been established in Tasmania from a single introduction of as few as 2 individuals (Schmid-Hempel et al. 2007).

The TMRCA analysis of subspecies B demonstrated that both clades B_1 and B_2 are ca. 67,000 years old and therefore have not diverged since its Australian introduction. Numerous locations have been isolated that contain the clade B_2 haplotypes, which geographically encompass a relatively large proportion of the European continent. This may make the final determination of the Australasian source populations difficult as it appears that thousands of years of human migration and trade throughout Europe and beyond has dispersed the differing subspecies and haplotypes widely. Our sampling scheme thus far has encompassed a large portion of the European continent with only Irish populations and those between Italy and Turkey remaining largely unanalysed. Surprisingly, none of the clades isolated in Australia were isolated in the United Kingdom, however, as these samples encompassed only a fraction of our sampling efforts in Europe, continued collection and analysis of samples from the United Kingdom is still needed. Therefore, the exact origins of the Australasian *F. auricularia* populations remain unknown.

Clearly, the reduction of genetic diversity in *F. auricularia* in Australia and New Zealand has not compromised it ability to successfully establish itself. *F. auricularia*'s invasion success can also be linked to anthropomorphic effects such as stratified dispersal events and the establishment of Europeanised environments in many temperate regions of Australasia. The ability of *F. auricularia* to adapt to the variable Australian climate has enabled it to establish across southern Australia within only xeric and sub-tropical climates being the points curbing its spread since being introduced into Tasmania over 170 years ago.

Acknowledgments

We wish to acknowledge the assistance of our collaborators Professor Thierry Wirth, Julliette Arabi and Alice Balard from the Muséum National d'Histoire Naturelle, without their assistance with this work would not have been possible. We also thank Svetlana Micic and Marc Widmer for the collection of the Western Australian samples. Finally, we wish to thank the Holsworth Wildlife Research Endowment for their financial support.

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Chapter 3 Predictive thresholds for forecasting the intraguild compatibility of *Forficula auricularia* and *Aphelinus mali* as biological control agents against woolly apple aphid in apple orchards

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Abstract

The woolly apple aphid (WAA), *Eriosoma lanigerum* is a well-known pest of apple orchards world-wide. Several natural enemies have been demonstrated to control WAA populations including the European earwig, *Forficula auricularia* and the WAA parasitoid *Aphelinus mali*. However, studies investigating these control agents individually have shown variable control of this apple orchard pest from season to season leading to chemical controls still being needed. We examine whether a beneficial interaction between *F. auricularia* and *A. mali* exists and calculate optimal beneficial numbers for each species for producers to target so as to achieve WAA control below spray thresholds. This was achieved by weekly of the abundances of WAA, earwigs, *A .mali* in 20 trees per site within organic, IPM and conventionally managed sites over two entire apple production seasons. We demonstrate that trees that possessed on average greater than 22 earwigs per week in traps located on the tree trunks within the first 7 weeks after blossom contained little to no WAA infestations, and that trees with between 14 and 22 earwigs on average per trap per week were observed to have WAA infestations well below spray thresholds. Where these targets were not met, a first generation of *A .mali* greater than 0.5 wasps per sticky trap per tree per week were required for acceptable WAA control to be achieved. If these beneficial insect targets were not met, WAA infestations covering > 30% of the tree occurred despite other predators being observed feeding on aphid colonies. Limited indirect evidence of possible intraguild predation of *A. mali* by earwigs was found, with instances of trees that contained high early season *A. mali* and 3rd instar earwig numbers having WAA infestations greater than those with fewer earwigs, indicating that the early season earwig population may be interfering with WAA control. Our findings suggest that if *F. auricularia* and *A. mali* numbers exceed these thresholds chemical intervention for WAA may not be required.

Keywords: *Forficula auricularia*, *Aphelinus mali*, *Malus domestica*, earwig, apple

Introduction

The woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausman), is a pest of pome fruit orchards and American Elm (Asante et al., 1991; Mols and Boers, 2001; Nicholas et al., 2005). During the apple growing season WAA forms conspicuous, densely packed colonies covered in a white, filamentous, waxy secretion on new growth and pruning cuts (Mueller et al., 1988). Severe WAA infestations can reduce tree health and vigour causing reductions in crop production (Goossens et al., 2011; Nicholas et al., 2005). In winter, nymphs and adults form hypertrophic galls on tree roots and limbs that reduce sap flow that can also rupture allowing fungal infections to occur (Asante et al., 1991; Gontijo et al., 2012; Nicholas et al., 2005). Additionally, these colonies are deemed to be a nuisance to fruit pickers, being messy and unpleasant to pick amongst and in extreme cases may cause respiratory issues when the waxy filaments are inhaled (Gontijo et al., 2012; Nicholas et al., 2005).

Like other aphid species, WAA exhibit rapid reproductive growth with up to 10-12 generations possible per apple growing season in warm climates (Goossens et al., 2011; Mueller et al., 1988), with each apterous virginoparae producing on average 100 nymphs within its lifetime (Asante et al., 1991). In many instances WAA infestations are controlled with the use of insecticide treatments (Goossens et al., 2011; Nicholas et al., 2005). However, due to increasing public awareness of the impacts insecticides have on both public and environmental health, a greater focus is being placed on the use of biological control agents such as predators and parasitoids (Suckling et al., 1999). A variety of species have been identified as predating on established WAA colonies including Syrphidae, Coccinellidae and Neuroptera (Asante, 1995; Bergh and Short, 2008; Gontijo et al., 2012). Despite being relatively effective at finding pre-existing WAA colonies their ability to prevent aphid outbreaks appears limited (Gontijo et al., 2012; Nicholas et al., 2005). Therefore efforts have turned to more localised predators to prevent this initial increase in aphid numbers from occurring there-by eliminating the lag in control observed with the aforementioned more mobile predators (Crawley, 1992).

One such localised, omnivorous predator that has been shown to predate WAA is the European earwig, *Forficula auricularia* L. (Carroll and Hoyt, 1984; Nicholas et al., 2005). Several studies have successfully shown natural and augmented earwig populations can significantly reduce WAA populations (Carroll and Hoyt, 1984; Mueller et al., 1988;

Nicholas et al., 2005). However, Carroll et al. (1985) found that the control exhibited by earwigs can vary from season to season, with adequate control observed one year but not in the following year possibly due to variable tree sizes or the availability of alternate food sources in larger tree canopies. However, it has also been suggested that as earwigs display a type II functional response as they appear to be unable to curb increasing WAA populations once a threshold of WAA has been surpassed (Asante, 1995).

Estimations of earwig abundance using traps situated on the tree trunks required for adequate aphid control have varied between studies. Nicholas et al. (2005) recommended that a seasonal mean between 4.98 and 8.30 earwigs per tree trap are required depending on the apple cultivar in question, whereas Mueller et al. (1988) recommended numbers between 3.7 and 7.3 earwigs per tree from mid to late summer. Despite this variability in WAA control, earwigs are deemed to be more effective aphid predators than other biological control agents such as ladybirds, lacewings and hoverflies (Nicholas et al., 2005). Indeed, Asante (1995) showed in laboratory based experiments that adult *F. auricularia* are able to predate up to 106 WAA within a 24 hour period with predation decreasing as aphid life stage increased.

One reason for this variability in WAA control is that earwigs display a complex life-cycle, which includes maternal care and aggregation behaviours followed by a seasonal dispersal soon after reaching adulthood (Hehar, 2007; Moerkens et al., 2009; Sauphanor, 1992; Walker et al., 1993). In late autumn, male and female earwigs form pairs in subterranean nests in preparation for overwintering as adults. Mating occurs early in autumn (Lamb, 1976) and continues through the overwintering phase (S. Quarrell, pers. obs.). Eggs are then laid from late winter to early spring, with males then aggressively evicted from the nest by the females soon after oviposition, after which time the males soon die (Gingras and Tourneur, 2001; Lamb, 1976; Lamb and Wellington, 1975). Female earwigs show strong maternal care for both eggs and young throughout the first nymphal instar until the end of the first moult, when both nymphs and females leave the nest to either nocturnally forage in trees and leaf litter, returning to the nest by day or leave the nest permanently (Kolliker, 2007; Lamb and Wellington, 1975). Dependent on subspecies of the earwig population in question the females will either then die (subspecies A) or will form another nest and lay their second, smaller clutch (subspecies A or B) (Lamb and Wellington, 1975; Wirth et al., 1998). Soon after the final nymphal moult, a decline in rapid earwig trap catches is observed (Moerkens et al., 2009). The reasons for this observed decline in earwig abundance as measured by catches in

traps are currently unclear as no evidence of dispersal, reduced food availability, increased natural enemy populations, disease or use of insecticides is evident (Moerkens et al., 2009). The decline in earwig abundance may reduce the ability of earwigs to control WAA infestations when the aphid's population growth rate is at its peak and may explain why adequate aphid control is not always observed.

Another invaluable natural enemy of WAA is the arrhenotokous parasitoid, *Aphelinus mali*. This parasitoid wasp, native to North America, has the potential to effectively control WAA with each female able to lay up to 85 eggs in its lifetime (Mols and Boers, 2001). However, due to *A. mali*'s later emergence from diapause than its host, its slow reproductive rate at temperatures less than 25 °C (Asante and Danthanarayana, 1992; Goossens et al., 2011), and lower reproductive capacity compared to WAA, they appear incapable of completely controlling WAA without assistance from other natural enemies or chemical intervention, especially in cooler climate apple growing regions (Asante and Danthanarayana, 1992; Goossens et al., 2011; Mols and Boers, 2001; Nicholas et al., 2005).

To date no studies have investigated the interplay of these two natural enemies with respect to WAA suppression. Goossens et al. (2011) examined the impact of *A. mali* on WAA field populations but did not account for predators. Similarly, Carroll et al. (1985) who augmented wild earwig populations and observed a lack of WAA control did not determine the *A. mali* population size, nor did Nicholas et al. (2005) during their observational study. However, Nicholas et al. (2005) did acknowledge that earwigs and *A. mali* may have a complimentary effect in successfully reducing aphid populations when observing the impact *A .mali* had on WAA under earwig exclusion.

In this study we evaluate the intraguild compatibility of *F. auricularia* and *A. mali* in achieving WAA control in apple orchards. This was achieved by weekly monitoring of the insect communities in apple trees with a history of WAA infestation, over two entire apple production seasons in 5 orchards that utilise differing management strategies (conventional, IPM and organic) to attain differing earwig, *A. mali* and WAA abundances. By doing so we aim to understand their respective population dynamics and accordingly develop predictive earwig and parasitoid monitoring thresholds, which producers can utilise to forecast the level of WAA infestation at the critical end of the apple production season.

Methods and Materials

To assess the impact earwigs and *A. mali* have on WAA populations, five apple orchards with varying management techniques within the Huon Valley, Tasmania, were selected so as to obtain a range of earwig, *A. mali* and WAA densities. The trials were run over two consecutive apple growing seasons commencing during blossom and concluding two weeks post-harvest. Season one commenced on the 28th October 2009 and ended on the 27th April 2010. Season two commenced on 19th October 2010 and concluded on 27th April 2011. Twenty apple trees (Fuji with MM106 rootstocks) from blocks with a history of WAA infestation were randomly selected from 5 orchards at the commencement of the trial (total n = 100). All trees were ca. 2 m high, spaced between 1.5 and 2.5 m apart and pruned to an open vase configuration. The orchards selected were two NASAA certified organic orchards Org1 (Lat. 43˚ 8.466' S Long. 146˚ 54.718' E), which applied no insecticide applications throughout the duration of the trial, and Org2 (Lat. 42˚ 59.755' S Long. 147˚ 4.328' E), which utilised mating disruption ties and applied *Bacillus thuringiensis* to control codling moth (*Cydia pomonella* L.) and light brown apple moth (*Epiphyas postvittana* Walker). Two IPM orchards that utilised visual and pheromone monitoring of Lepidopteran pests, natural enemies and the minimal use of targeted chemical insecticides for pest insect control (IPM1 (Lat. 43˚ 8.485' S Long. 146˚ 53.863' E) and IPM2 (Lat. 43˚ 8.612' S Long. 146˚ 55.003' E)). These IPM sites applied targeted applications of chlorpyrifos to manage apple looper (Geometridae) outbreaks during the trial. The final orchard, Con1 (Lat. 43˚ 1.080' S Long. 147˚ 3.833' E) was conventionally managed and utilised calendar spray applications of systemic broad-spectrum insecticide (thiacloprid) in the 9th week of each season to control *C. pomonella, E. postvittana* and WAA. All orchards utilised fungicides to control apple scab (*Venturia inaequalis* Cooke) as per standard practice with the Org using lime only, Org2 using lime sulphur and the IPM and conventional sites using rotations of Dithianon and Difenoconazole. The organic sites maintained high levels of groundcover under the trees, the IPM sites utilised a moderate to low level of groundcover and the conventional site maintained minimal groundcover under the trees.

Earwig populations were monitored using corrugated cardboard rolls (8.5 cm x 9 cm) attached with garden twine (Zenith, REA 0060), at the base of each tree 30 cm above ground level. The number, sex and life stage of each earwig found in the cardboard rolls was recorded weekly and subsequently trapped individuals were released at the tree base. The

earwig traps were replaced weekly to prevent the presence of any aggregation pheromones from impacting earwig population monitoring (see chapter 5). WAA levels were visually graded categorically between 0 and 5 (modified from Nicholas et al., 2005). Ratings were: 0 = no aphids; 1 = < 5% limb coverage; 2 = 5-10% limb coverage colonies; 3 = 11-30% coverage; 4 = 31-50% coverage; 5 = > 50% coverage on all limbs (Nicholas et al., 2005). Only live WAA infestations were scored, any aphid mummies parasitised by *A. mali* were excluded for aphid scores. To determine the population sizes of other insects within the tree canopies, including *A. mali*, a single adhesive insect trap made from yellow corflute (250 x 105 mm) coated with Tanglefoot® was placed on a branch, 1.5 m above ground level on each monitored tree. The yellow adhesive insect traps were changed weekly by covering them in cling film, returning them to the laboratory and storing them at -12 ˚C until insect identification occurred.

The abundance and diversity of the arthropods caught on the yellow adhesive traps were recorded. Numbers of *A. mali* were recorded separately on each trap. All other taxa were identified to order and placed into functional feeding groups characterised as; predators (insects that consume other insect species e.g. Coccinellidae and Neuroptera), herbivores (consumption of apple foliage, fruit and sap feeders), parasitoids and neutrals (none of the above). Weather data (weekly minimum/maximum temperatures and rainfall) were collected from nearby Bureau of Meteorology weather stations within 2 km of the experimental sites, situated at Grove Research Station (Lat. 42˚59.0' S Long. 147˚ 4.583' E) and Geeveston (Lat. 43˚ 9.600' S Long. 146˚ 55.200' E).

Statistical analysis

To assess the impact early season *F. auricularia* and *A. mali* have on the level of WAA infestation observed in orchards recursive partitioning analysis was conducted. Recursive partitioning develops conditional inference trees. At each step a null hypothesis of no association is tested between the outcome and the covariates with the process stopping if the null hypothesis is retained. If the null hypothesis is not retained the covariate with the strongest association is used to split the data into disjoint sets. This process is repeated until no covariate is associated with the data set (Strobl et al., 2009). The following variables were included in the recursive partitioning models; management type (conventional, IPM and organic), the mean number of first generation *A. mali* per orchard per season (A. mali_1st generation), which were deemed to be those *A. mali* trapped within the first 4 weeks post-

blossom (Goossens et al., 2011), the mean number of earwig adults (adults_1st7weeks), 4th instars (4th instar_1st7 weeks) and the total number of earwigs (total_earwigs_1st7wks) observed within the first quarter (7 weeks) of the field season per orchard, the mean first generation 2nd instars earwigs (2nd instars_1st generation) and 3rd instar earwigs per tree (3rd instars_1st generation). These earwig generation sizes were determined by identifying the beginning and the end of each generational peak. The WAA scores observed in each tree after week 8 through to the end of each season were used as the dependent variable for all models. To account for the presence of alternative prey items for the earwigs other than WAA, the mean number of herbivores observed on the sticky cards within the first quarter (7 weeks) of each season, in each orchard was also incorporated into the model. All data analysis was performed with R version 2.15.1 using the "party" package and the "ctree" function for the recursive partitioning. The differences in arthropod abundance within orchards between years were assessed using Wilcoxon Sign rank tests using IPM SPSS Statistics version 19.

Results

Phenology and population dynamics

WAA

Using weather station data and the WAA models developed by Asante *et al.* (1991) and validated by Goossens *et al.* (2011) we predict that WAA went through ca. 5-6 generations per apple growing season during both the 2009/10 and 2010/11 seasons. WAA scores differed significantly between years at all sites except for one of the IPM sites (Figure 3-1; Wilcoxon Sign Rank; IPM1: $Z = -1.89$ $P = 0.059$; IPM2: $Z = -5.10$ $P < 0.001$; Org1: $Z = -13.00$ $P < 0.001$; Org2: $Z = -6.19$ $P < 0.001$; Con1: $Z = -6.18$ $P < 0.001$). At both IPM orchards WAA scores well below spray thresholds were recorded throughout the season. The presence of WAA was not recorded at either of the IPM sites prior to the application of chlorpyrifos in either year (Figure 3-1). In IPM1, no aphid colonies were observed at the end of the 2009/10 and 2010/11 seasons. In IPM2, the final WAA scores were (mean ± SEM) 0.1 ± 0.1 at the end of both seasons. At Con1, moderate to low end of season WAA control was observed during the 2009/10 season with a mean WAA score of 2.5 ± 0.2 (max score = 3) whereas at the end of the 2010/11 season lower level infestations were observed (mean 1.6 ± 0.2, max = 3). At Org1, during the 2009/10 season the WAA infestation levels reached an unacceptable mean score of 4.7 ± 0.2, which led to the suppression of fruit bud development and reduced production in the following year (S. Quarrell, pers. obs.). During the 2010/11 season, WAA scores at Org1 reached a mean score of 1.7 ± 0.1 by week 12, however, by the

end of the season adequate WAA control was achieved without chemical intervention (mean score = 0.1 ± 0.1). At Org2, at the end of the 2009/10 season, low levels of WAA infestation were observed (0.6 ± 0.2) despite some individual trees showing moderate infestations (max = 3).

Earwigs

Early season earwig trap catches during both the 2009/10 and 2010/11 field seasons showed populations to contain few adults (all from the previous season) of which a greater number were adult females (Figure 3-2). No adults were caught after week 2 of the 2009/10 season and after 3 week in all orchards during the 2010/11 season with the exception of Org1 where they were trapped until week 4. Two distinct generations of juveniles were observed at all orchards in both years, demonstrated by the two peaks in the trap catches of 2nd and 3rd instars prior to week 14 during both seasons (Figure 3-2). The consistent laying of two clutches per season is characteristic of subspecies B in *F. auricularia* (Wirth et al., 1998). Earwig trap catches were variable between both orchards and years (Figures 3-1 and 3-2). During both seasons the lowest peak trap catches were observed at Con1 (mean ± SEM; 2009/10: 10.2 ± 2.2, 2010/11: 7.3 ± 1.6). The highest trap catches were observed at IPM2 (2009/10: 57.6 ± 3.7, 2010/11: 46.2 ± 5.7). Moderate to high trap catches were also observed at the other sites (IPM1 2009/10: 29.9 ± 2.8, 2010/11: 33.7 ± 3.7; Org1 2009/10: 17.4 ± 2.3, 2010/11: 22.5 ± 2.0; Org2 2009/10: 29.7 ± 3.8, 2010/11: 26.5 ± 3.5). The timing of these maximum catches also varied between seasons and orchards with maximum catches ranging between weeks 5 through 10 during the 2009/10 season and 7 and 14 during the 2010/11 season (Figure 3-2). The maximum traps catches in all instances contained 2nd, 3rd and 4th instar juveniles. As the 4th instar juveniles passed through their final moults, trap catches at all sites were observed to decline rapidly (Figure 3-2).

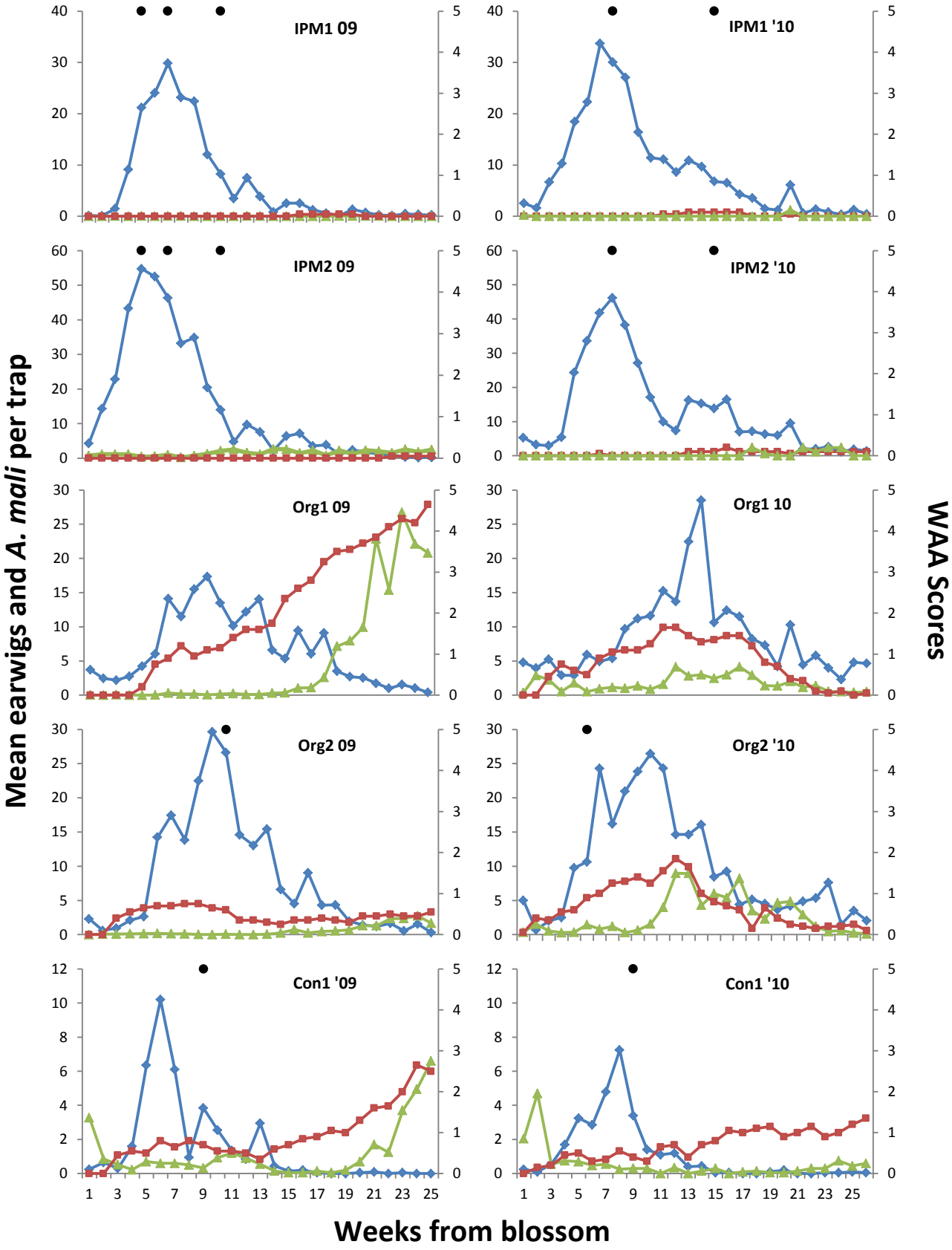

Figure 3-1. Mean *Forficula auricularia* (blue) and *Aphelinus mali* (green) captured and WAA scores (1-5) per trap per tree (red) from organic (n = 2), IPM (n = 2) and conventionally managed (n = 1) orchards through 2009/10 (left) and 2010/11 (right) apple production season in Tasmania, Australia. Black dots above figures indicate timing of insecticide applications.

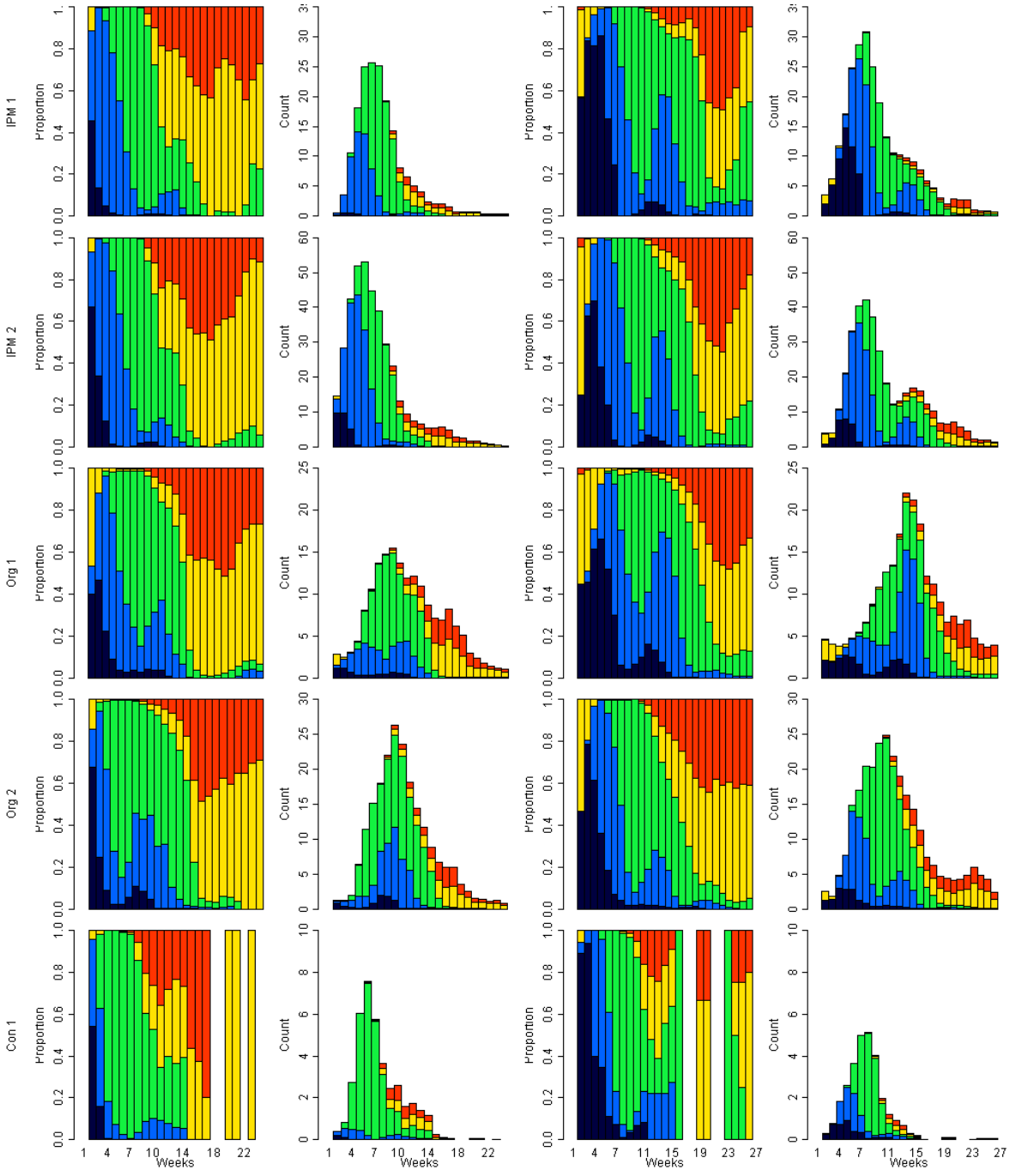

Figure 3-2. Distribution of the mean proportions and mean counts of 2nd instar (black), 3rd instar (blue), 4th instar (green), adult male (red) and adult female (yellow) *Forficula auricularia* by weeks observed with earwig traps (n = 20) located on the tree trunks for each orchard over the 2009/10 (left) and 2010/11 (right) apple production seasons. Population data was smoothed by using a 3 week running mean.

Aphelinus mali

Significantly larger first generation *A. mali* numbers were observed at the beginning of the 2010/11 season compared to the 2009/10 season at both the organic and the conventional orchards (Table 3-1), but not at IPM2 where larger numbers were observed at the beginning of the 2009/10 season. Due to the low levels of WAA infestation at the IPM sites extremely low numbers of *A. mali* were observed during both the 2009/10 season (mean ± SEM, IPM1: 0.01 ± 0.01; IPM2: 1.3 ± 0.1) and the 2010/11 season (IPM1 0.1 ± 0.0; IPM2 0.01 ± 0.01). Due to these low numbers of *A. mali* no significant difference was observed at IPM1 (Table 3-1, Figure 3-1).

At Org2, relatively low first generation *A. mali* numbers were observed per tree in both years (Figure 3-1; mean ± SEM; 2009/10: 0.1 ± 0.0; 2010/11: 0.7 ± 0.2). At the end of the 2009/10 season low levels of WAA infestation were observed (0.6 ± 0.2) despite some individual trees showing moderate infestations (max = 3). The low WAA infestations observed at the end of the 2010/11 season appear to have been due in part to several large *A. mali* emergences, which were observed during the 2010/11 season in weeks 12, 13, 18 and 22 yielding mean (± SEM) sticky traps catches of 8.45 (± 1.3), 8.2 (± 4.4) and 4.65 (± 2.9) wasps per tree respectively. These flights agree with the day degree models developed for *A. mali* by Asante and Danthanarayana (1992) utilising the date of first emergence (week 2) and weather station data suggested that *A. mali* went through ca. 4-5 generations per year in the monitored orchards during both the 2009/10 and 2010/11 seasons, with observed flights recorded within 1 week of those predicted. These emergences coincided with reductions at Org2 in WAA infestation with a final score of 0.1 ± (0.1) recorded in week 28 (max = 2).

Table 3-1. Mean (± SEM) first generation size of *A. mali* observed collected from sticky traps in 20 trees in 5 orchards during the 2009/10 and 2010/11 apple production seasons. Statistics conducted using Wilcoxon Sign Rank test.

	Season			
Orchard	**2009/10**	**2010/11**	***Z***	***P***
IPM1	0.01 (0.01)	0.01 (0.01)	0.00	1
IPM2	1.29 (0.09)	0.00 (0.00)	- 7.17	< 0.001
Org1	0.05 (0.03)	1.66 (0.24)	- 6.24	< 0.001
Org2	0.11 (0.04)	0.70 (0.20)	- 3.60	< 0.001
Con1	1.43 (0.22)	2.13 (0.25)	- 2.63	0.008

Other herbivores and predators

The mean density of predators other than earwigs and herbivores other than WAA at each orchard varied between years and management type (Table 3-2). Few commonly regarded aphid predators such as the common spotted ladybird, *Harmonia conformis* (Boisduval), Neuroptera (Chrysopidae or Hemerobiidae) and Syrphidae were caught on the sticky cards used to monitor the insect populations, despite their adult and larval stages occasionally being observed feeding on WAA (S. Quarrell, pers. obs.). However, large numbers of predatory Diptera including Dolichopodidae and Empididae were captured. The mean density of herbivores at each orchard varied between years and management type being especially high in organic orchards (Table 3-2). Herbivores were observed to increase throughout both seasons with large numbers of herbivores dominated largely by the apple leaf hopper (*Edwardsiana australis* Baker) captured on the sticky cards in the last week of each observation season.

Table 3-2. Mean (± SEM) herbivore and predator sticky trap catches from 5 orchards collected over the 2009/10 and 2010/11 apple growing seasons. Statistics conducted using Wilcoxon Sign Rank test.

	Herbivores				**Predators**			
	Season				**Season**			
Orchard	**2009/10**	**2010/11**	***Z***	***P***	**2009/10**	**2010/11**	***Z***	***P***
IPM1	2.1 (0.2)	3.2 (0.3)	- 4.80	< 0.001	1.5 (0.1)	2.0 (0.1)	- 4.20	< 0.001
IPM2	1.6 (0.1)	7.4 (0.7)	- 7.52	< 0.001	1.7 (0.1)	2.2 (0.1)	-3.37	0.001
Org1	23.6 (1.6)	25.3 (1.7)	- 3.51	< 0.001	5.1 (0.3)	4.7 (0.2)	- 0.13	0.899
Org2	28.0 (1.7)	72.9 (4.3)	- 9.99	< 0.001	1.7 (0.1)	1.4 (0.1)	- 2.67	0.008
Con1	1.3 (0.1)	1.7 (0.2)	- 1.57	0.116	0.5 (0.0)	0.3 (0.0)	- 3.22	0.001

Predictive thresholds for WAA management

Management of orchards was the highest predictor of WAA infestation, with IPM being split away from the organic and conventional orchards (Figure 3-3, Node 1). The IPM managed orchards are divided by having a mean 4^{th} instar earwig trap catches greater or less than 0.4 earwigs per trap per week within the first 7 weeks after blossom, though there is no clear difference in WAA infestation (Terminal Nodes 14 and 15). The organic and conventional orchards are split (Node 2) by whether sticky trap catches caught a mean of greater than 4 predators per week (i.e. Neuroptera and Coccinellidae). Those trees with greater than 4 predators and *A. mali* at densities less than 0.5 wasps per sticky trap per week possessed the highest WAA infestations (mean score = 4) indicating *A. mali* does aid in the reducing WAA

numbers. Following the impact of high predator and high *A. mali* numbers, 3rd instar earwig catches had the next greatest impact on WAA counts with 3rd instar earwig trap catches per week of > 10 possibly interfering with WAA control in a small number of instances (Terminal Node 12, n = 46, mean score = 1). However, in orchards with low predator numbers (< 4 per trap per week), 4th instar earwig numbers less than 9 earwigs per trap per week and > 0.5 wasps per sticky trap per week, WAA numbers were reduced to below spray thresholds (Terminal Node 6, n = 1291, mean score = 1).

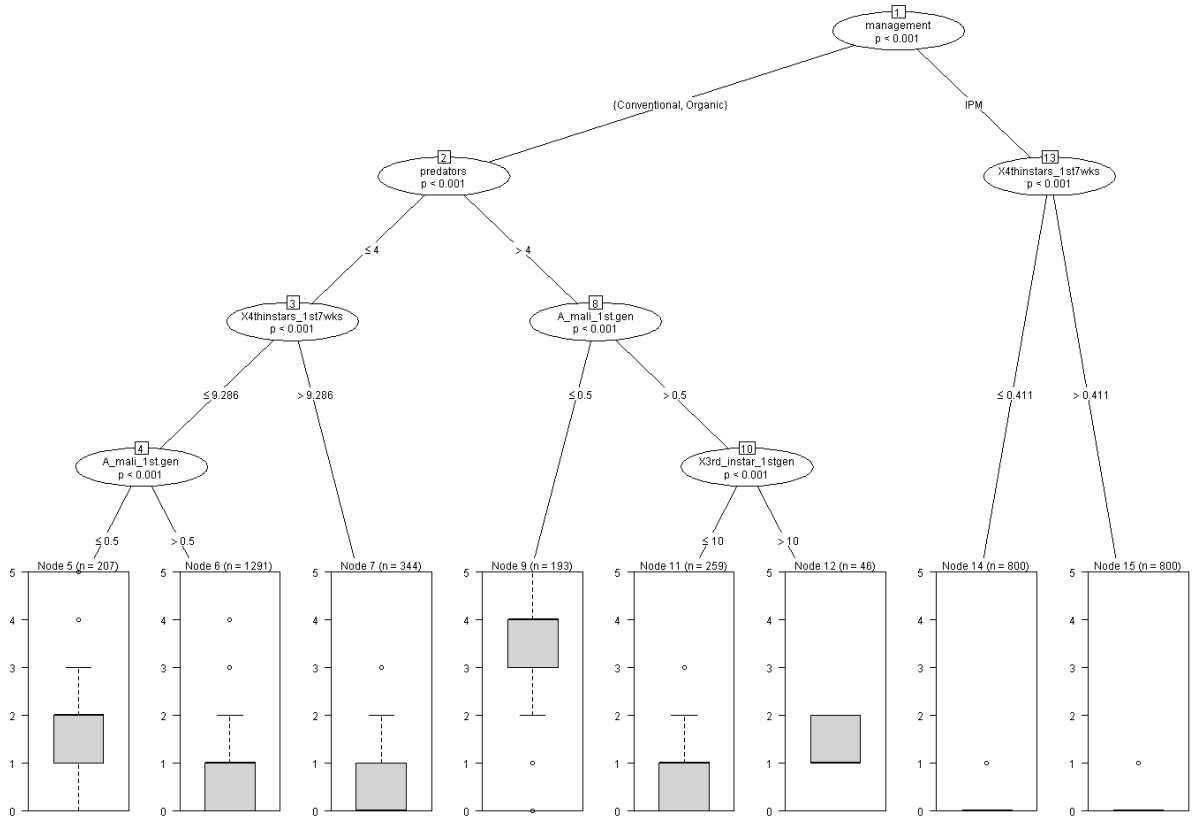

Figure 3-3. Conditional inference regression tree indicating the differences in the level of WAA infestation observed throughout the last three quarters of two consecutive apple production seasons with respect to orchard management type, mean predator and herbivore numbers, 4th instar *Forficula auricularia* observed in the first quarter of each apple production season and first generation trap catches of 2nd instar and 3rd instar *Forficula auricularia* and *Aphelinus mali*.

The impact of earwigs and *A. mali* on WAA scores is clear when management and other predators are removed from the model (Figure 3-4). The first predictor of WAA scores is the mean total number of earwigs caught per tree (irrelevant of life stage) over the first 7 weeks after the commencement of blossom, where a mean total greater than 15 earwigs per trap per week leads to low WAA scores. Furthermore, if the total earwig count exceeds 22 earwigs

per trap per week then a mean WAA score of zero will eventuate (Terminal Node 7, n = 1600, mean score = 0). However, if the total number of earwigs per trap per week during this first 7 week period does not exceed 15 earwigs then the next predictor is the size of the first generation of *A. mali* caught on sticky cards (Node 2). If the mean number of the *A .mali* first generation is low (< 0.05 wasps per sticky trap per week) then WAA scores will exceed spray thresholds at the end of the season (Terminal Node 3, mean score = 3). Conversely, if *A. mali* numbers exceed 0.5 wasps per sticky trap per tree but the total earwigs numbers are below 15 earwigs per trap per week then reasonable control can still be achieved (Terminal Node 4, mean score = 1).

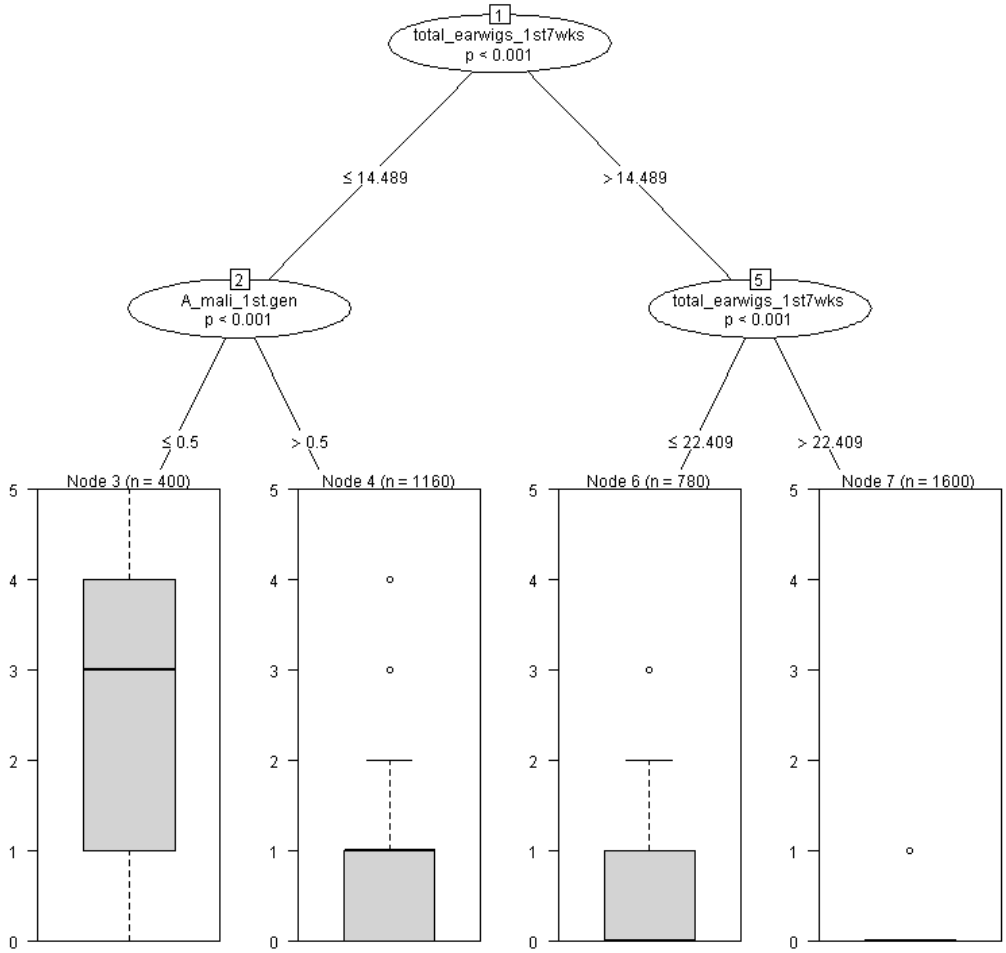

Figure 3-4. Conditional inference regression tree indicating the differences in the level of WAA infestation observed throughout the last three quarters weeks two consecutive apple production seasons with respect to the number of herbivores, total and 4th instar *Forficula auricularia* observed in the first quarter of each apple production season and first generation trap catches of 2nd instar and 3rd instar *Forficula auricularia* and *Aphelinus mali*.

Discussion

During this study few adult earwigs were trapped at the beginning of each season at all orchards. Of these adults, few males were observed, most likely due to over-wintering mortality or mortality following eviction from the nest by the female which, is commonly observed in *F. auricularia* (Gingras and Tourneur, 2001; Lamb, 1976). As *F. auricularia* in Australia are subspecies B (two generations per year) (Wirth et al., 1998) the females observed, rapidly disappeared within the first 2-4 weeks of each season most likely establishing new nesting sites for their second clutch of eggs. The largest trap catches occurred after this time and consisted of first generation juvenile life-stages except first instars, which remain within the nest until they reach the second instar.

The size of the first generation of *A. mali* varied between orchards and years with sites Con1 and Org1 containing the largest early season numbers. Indeed, this observation in the second year agrees with that of Goossens et al. (2011) who also noted that orchards which possessed large infestations of WAA in the previous year contained larger early season *A. mali* in the following year. However, despite possessing the largest first generation *A. mali* populations, WAA infestations reached unacceptable levels when earwig numbers were also initially low. This indicates that despite their relatively high reproductive capacity *A. mali* appears unable to solely control WAA, as has also been reported elsewhere (Mols and Boers, 2001). It therefore appears crucial that an alternative predator such as *F. auricularia* be present early in the season before WAA are able to become well established.

Indeed, our models indicate that for effective WAA control to occur over the entire growing season, large early season earwig numbers are required, with a minimum of 15 earwigs per trap per week needed within the first 7 weeks after blossom. If these earwig numbers were not present then a minimum of one *A. mali* in every second sticky trap per week was desirable, or adequate control may not be observed. It should be noted that these estimates require field validation and may not eliminate the need for ongoing monitoring. These earwig numbers are at least twice those recommended by both Nicholas et al. (2005) and Mueller et al. (1988) who recommended either seasonal or mid-summer means between 3.7 to 8.3 earwigs per tree. Our estimates are based on a predictive model and not seasonal means (Nicholas et al., 2005), or mid-season estimates (Mueller et al., 1988) and therefore would be higher due to the seasonal decline observed in other earwig population studies (Moerkens et

al., 2009). However, if WAA are successfully established within the first 7 weeks post-blossom without the earwig and parasitoid estimates being met, it appears unlikely that the presence of these two natural enemies will contain WAA as was observed at Org1 during the 2009/10 season. This result may also explain the lack of WAA control observed by Carroll et al. (1985) who attempted to augment natural earwig populations for control of pre-existing WAA colonies.

These elevated earwig numbers within the first 7 weeks of the growing season are largely comprised of early season, first generation juvenile earwigs. This demographic would be expected to have the greatest potential to suppress WAA, not only due to their high population density, but also their physiological requirement to consume high levels of prey, in order to receive protein rich diets sufficient to maximise growth and development (Boukary et al., 1998b). Indeed, it has been demonstrated that juvenile male *F. auricularia* fed higher protein diets are more sexually competitive than individuals fed on level protein diets (Tomkins, 1999) and though not studied in this species it is commonly known that females in many species require protein rich diets during their juvenile stages to ensure maximal egg development during adulthood (Boukary et al., 1998a; Jalali et al., 2009; Mahdian et al., 2006; Wheeler, 1996). This predatory feeding would effectively suppress WAA infestations to levels until parasitoid populations are at numbers sufficient to assume control. However, this increased level of predation may have a negative effect on the early season parasitoid populations. Indeed, our models do indicate that some intraguild predation may have occurred in a few instances (Figure 3-3) where trees, which contained greater than 0.5 *A. mali* per trap and a first generation of 3rd instar earwig trap catches greater than 10 earwigs contained marginally higher WAA infestations than those with fewer 3rd instar earwigs. The impact of intraguild predation is possibly lessened due to earwigs being a generalist omnivore and therefore most likely feeding on a variety of food resources compared to specialist, aphidophagous predators such as Coccinellidae (Xue et al., 2012). However, as our models demonstrate early season *A. mali* do impact on the level WAA control at the end of the season, their predation even at low levels by earwigs at the beginning of the season is important to the subsequent population dynamics.

To attain differing abundances of earwig and *A. mali*, commercial orchards with differing management strategies and hence insecticide usage, though none specifically targeting WAA, were utilised. Insecticides used included the use of chlorpyrifos at the IPM sites for apple

looper and *Bacillus thuringiensis* at Org2 to control *C. pomonella* and *E. postvittana*. The timing, application and toxicity of these insecticides used appear to have had little impact on either WAA, *A. mali* or earwig numbers at either the IPM or organic sites. Although chlorpyrifos is highly toxic to WAA (Nicholas et al., 2003) and earwigs (Bower 1992) its application at the IPM sites was made when no WAA colonies were observed (prior to week 5 in 2009/10 or week 8 in 2010/11) (see figure 3.1) and appeared to have no discernable impact on the earwig population. Similarly, the *Bt* application used to control Lepidopteran pests at the organic site Org2, also appears to have little impact on any of these insect species. Similarly, thiacloprid, which was sprayed at Con1 in both years is known to have little impact on WAA, but is known to impact *A .mali* (Kim et al., 2009) and *F. auricularia* (Shaw and Wallis 2010). However, this site was selected due to it containing few natural enemies and a history of WAA infestation and therefore the application of thiacloprid should not impact on the legitimacy of the models generated here.

The earwig trap catches were observed to decline steadily once adulthood had been reached at all sites. Moerkens et al. (2009) postulated that this decline was a due to density dependent factors such as food availability, pathogens and parasites and the use of insecticides. However, alternate prey resources were observed to increase throughout both seasons in or study with large numbers of insects including the apple leaf hopper (*E. australis*) captured on the sticky cards. It is therefore unlikely that the observed decline in earwig trap catches in this and other earwig studies is due to a decline in food resources. Similarly, we observed earwigs infected with the fungal pathogen *Beauveria bassiana* and parasitic nematodes during this study, but their presence was in very low levels and therefore unlikely to drive such a decline in trap catches. The use of insecticides also appears to be an unlikely cause as the decline was observed at all sites including Org1, where no insecticide treatments were applied throughout either season. However, the decline does appear to have been more rapid at Con1 after the annual application of a systemic insecticide. It appears therefore that this decline in earwig trap catches is due to other factors which possibly include the formation of mating pairs earlier in the season than previously reported, which would also explain the relatively low number of adults observed after the final juvenile moult. Alternatively, the earwigs may have remained within the tree canopies, utilising the apple bunches as daytime residences. If this is the case large earwig numbers would not be observed in the cardboard earwig rolls situated on the tree trunks. Indeed, some earwigs were observed in the fruit bunches (S. Quarrell, pers. obs.), however; the numbers observed appear insufficient to account for the decline in trunk

trap catches. This is further supported by only a marginal increase in trap catches observed after apple harvest when these alternative daytime residences are removed.

The movement of other more mobile predatory insects, such as lacewings and ladybirds into tree canopies where WAA were already present, was observed during this study. However, these predators, though important in aphid control, were only observed in the aphid infested canopies after the WAA had reached potentially problematic levels. This highlights the importance of polyphagous or omnivorous, localised predators such as earwigs, which can subsist on plant matter or non-pest prey when pest densities are low. These predators can suppress the pest's rapid growth phase or prevent the reinvasion of pest species, thereby preventing problematic infestations from occurring. In comparison, more mobile specialist predators such as Coccinellids may require high pest densities to locate prey items in dispersed landscapes. This enables the pests to establish, effectively creating a lag phase between pest and predator establishment (Symondson et al., 2002), which may not be deemed an acceptable scenario by many producers. Similarly, an overreliance on specialist parasitoids such as *A. mali*, may also lead to a scenario where unacceptable aphid infestations may occur before control maybe apparent.

Conclusions

We demonstrate that the generalist predator, *F. auricularia* and the specialist parasitoid, *A. mali* can prevent problematic WAA infestations from occurring. Our analysis shows that apple trees, with early season earwig trap catches greater than 22 individuals per week, should have low to no WAA infestation at season's end. However, if these earwig numbers are not present a first generation of *A. mali* greater than 0.5 wasps per sticky trap per week should be sufficient to suppress WAA without chemical intervention. In the event that earwig and parasitoid numbers are both below these thresholds, then chemical intervention may be required.

Acknowledgments

We wish to thank the apple producers John Evans, Simon Burgess, Andrew Smith and Howard Hansen who generously provided their time and resources. We also wish to thank Shasta Jamieson for her assistance with the counting of sticky card trap catches and Dr. Ross

Corkrey for his assistance with the statistical analysis. This work has been supported by grant funding from Horticulture Australia Limited (MT 09006).

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Chapter 4 Cherry damage and the spatial distribution of the European earwig, *Forficula auricularia* in sweet cherry trees

Formatted for the journal "Bulletin of Entomological Research"

Abstract

The European earwig, *Forficula auricularia* is an invasive insect pest found in many temperate regions of the world. Despite being well known predators in apple orchards, they are considered pests in sweet cherry though this has never been empirically tested. The aim of this study was to quantify earwig presence and spatial distribution in cherry tree canopies and examine how these factors impact on any fruit and stem damage observed in cherry varieties Ron's Seedling, Lewis, Sweet Georgia and Lapin. Cherry bunch size, bunch position along the limb, limb aspect and their relationship to both earwig presence and cherry damage were also examined. Significant differences in the type and frequency of earwig damage were observed between varieties with earwig exclusion reducing fruit damage up to 9-fold and stem damage up to 5-fold. In Ron's Seedling, cherry stems were 40 times more likely to be damaged than Lewis stems and Lewis fruit was twice more likely to be damaged than Ron's Seedling fruit. Similarly, Sweet Georgia fruit were 4.5 times and stems 5 times more likely to be damaged than Lapin fruit cherries. Earwigs were strongly aggregated within cherry bunches with larger bunches typically occurring in the outer third of the tree limbs. Greater earwig numbers and damage were observed in larger bunches in all varieties except Ron's Seedling where stem damage occurred irrelevant of bunch size. No predictive relationship between earwig numbers in trunk traps at harvest nor found within the tree canopies at harvest and the level of cherry damage could be found.

Keywords Earwig, Dermaptera, *Forficula auricularia*, sweet cherry, *Prunus avium*

Introduction

The European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) is a subsocial, invasive insect species found in many temperate regions around the world (Lamb & Wellington 1975). During its seasonal activity window *F. auricularia* exhibit a strong thigmotactic response, aggregating in large numbers under rocks, logs and within tree canopies aided via the use of a putative aggregation pheromone (Helsen *et al.* 1998; Sauphanor 1992; Walker *et al.* 1993). Despite their invasive nature earwigs have been shown to be useful biological control agents against numerous insect pests in apple (Carroll & Hoyt 1984; Dib *et al.* 2011; Nicholas *et al.* 2005) and orange orchards (Piñol *et al.* 2010), hop gardens (Buxton & Madge 1976) and kiwi fruit (Logan *et al.* 2011). However, due to their omnivorous feeding habit they have also been long considered an urban (Lamb & Wellington 1975; Walker *et al*. 1993) and agricultural pest in many vegetable (Rentz & Kevan 1991) and soft-fleshed fruit crops such as apricots, where earwigs have been reported to damage up to 40% of some apricot harvests (McLaren 1999).

In sweet cherries (*Prunus avium* L.), earwigs are regarded as a pest reportedly damaging fruit and are a potential issue in post-harvest packing, export and biosecurity (Bower 1992). The impact *F. auricularia* has on cherry production is currently unknown, although in extension literature, damage attributed to earwigs includes cherry leaf, fruit bud, pedicel (henceforth referred to as stem) and fruit damage in Australia (Bower 1992; Domeney & Williams 2002) and in the U.S.A. (Grant *et al.* 2005). This literature states earwig feeding results in shallow, irregular holes in the cherry fruits, which may also become infected with secondary fungal infections (Hetherington 2005).

Despite its assumed pest status there has been no empirical research undertaken quantifying the impact earwigs have on cherry production or any action thresholds developed to determine insecticide usage in cherries. A web-search of university and governmental agricultural extension services found numerous documents stating that *F. auricularia* is a pest in cherries and provides chemical management strategies for their control (Antonelli 2006; Bower 1992; Domeney 2009; Grant *et al.* 2006; James 2011). It is therefore essential that any impact that earwigs may have on cherry production be quantified to determine whether these anecdotal reports are accurate, particularly as broad-spectrum insecticide applications remain the primary methods of earwig control.

The study firstly aims to examine how intra-tree factors including cherry bunch size, cherry bunch position along the limb and limb aspect influence earwig location in the tree canopy. By excluding earwigs from limbs we also aim to quantify cherry fruit and stem damage by earwig feeding. Secondly, we examine how any level of damage found varies according to both the aforementioned intra-tree factors and the cherry varieties Lapin, Lewis, Ron's Seedling and Sweet Georgia in two regions of Australia. Finally, we explore whether there is a relationship between catches of earwigs in trunk traps at harvest and the level of earwig damage found in cherry trees.

Methods and Materials

Experimental study sites

Exclusion and cherry bunch size experiments were undertaken in three cherry orchards across New South Wales (NSW) and Tasmania (TAS) Australia, all of which were known to contain large earwig populations (Table 4-1). In Young, NSW on one property two blocks were selected one of Ron's Seedling (RS1: 34˚ 18.296′ S 148˚ 21.042′ E) and one block consisting of alternating plantings of Ron's Seedling and Lewis cherry trees (RS/LW). On a second nearby property a single block of Ron's Seedling was selected (RS2: 234˚ 26.877′ S, 148˚ 18.974′ E). In Grove, Tasmania (TAS) one block of Lapin and one block of Sweet Georgia were selected from a NASAA certified organic orchard (42˚ 59.755' S, 147˚ 4.328' E). All cherry trees were pruned to a vase system. No chemical insecticide applications were applied over the experimental period. Row orientation, row and tree spacing, tree age and ground cover all varied between blocks (Table 4-1).

Table 4-1. Experimental site characteristics for the *Forficula auricularia* exclusion and cherry bunch size experiments.

	Experimental Block				
	RS1	RS2	Lapin	RS/LW	Sweet Georgia
Experiment	Exclusion	Exclusion	Exclusion/Bunch size	Bunch size	Bunch size
Trees sampled	20	20	20	40	20
Data collected	16th Nov 11	15th Nov 11	9th Jan 12	17th Jan 11	14th Jan 12
State	NSW	NSW	TAS	NSW	TAS
Planting date	1999	1996	2002	1983/1988	2006
Row orientation	N/S	E/W	NW/SE	E/W	NW/SE
Row spacing (m)	5.50	6.10	3.50	6.70	3.50
Tree spacing (m)	2.30	3.80	1.25	3.35	1.25
Irrigation	drip	nil	drip	nil	drip
Management type	conventional	conventional	organic	conventional	organic
Ground cover	mulch	mulch	grass	mulch	grass
Bird netting	no	no	yes	no	yes
Rain covers	yes	no	no	no	no

Earwig exclusion and mapping earwig, cherry bunch size and cherry damage within the canopy

Three blocks (RS1, RS2 and Lapin) were used for this experiment (Table 4-1). Three weeks before fruit harvest one limb from each of 20 trees to be sampled per block was randomly designated as an exclusion limb and acted as a control for any damage that occurred in the absence of earwigs. An exclusion band was applied to each exclusion limb by wrapping 5 cm wide duct tape around the limb's base and then smearing Tanglefoot® over the tape to prevent earwigs accessing the developing fruit on the limb. Any earwigs and damaged fruit found within cherry bunches on this exclusion limb were removed at this time. To monitor earwig numbers at harvest an earwig trap consisting of a rolled piece of corrugated cardboard (8.5 cm x 9 cm) was tied with garden twine (Zenith, REA 0060) to each of the 20 tree trunks 30 cm above ground level. To assess the efficacy of the exclusion band another earwig trap was also tied above the limb's exclusion band. These exclusion limb traps were checked for earwigs one day after trap placement and any earwigs released at the base of the tree and for a second time cherry bunches were checked for damaged fruit and earwigs found in any bunches on the exclusion limbs removed.

Sampling for earwigs and cherry damage was done a maximum of two days prior to cherry harvest (Table 4-1). At this time, the number of earwigs found within the trunk and exclusion

limb traps, their sex and life stage, number of cherry bunches per limb, number of cherries per bunch, damaged cherries per bunch, damage type and earwigs found within each bunch were recorded on the exclusion limb and four limbs randomly selected from each of the four cardinal points (North, South, East and West). Earwig damage was initially determined by confining earwigs in plastic containers in the laboratory and observing the resulting damage. Earwig damage type was characterised as either 1) fruit damage - chewing damage to the cherry fruit (Figure 4-1A) or 2) stem damage - chewing damage to the cherry fruit stem (Figure 4-1B). The presence of other chewing insects found within cherry bunches including Curculionidae and *Carpophilus* beetles, which may have caused any observed damage, were also recorded. The position of each cherry bunch along the limb was recorded by allocating each as being in the low, middle or high (terminal) third of the limb and as either on the main limb, fork shaped limb or on a small side branch.

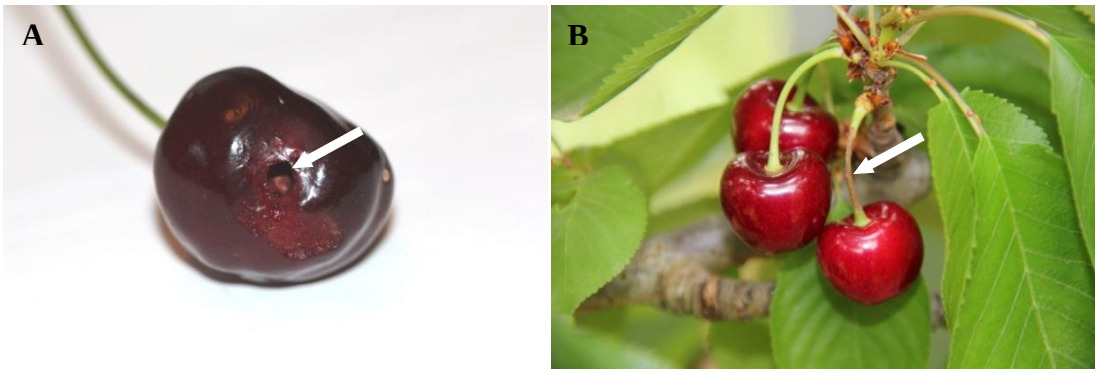

Figure 4-1. (a) Severe cherry *Forficula auricularia* fruit damage on Lapin cherry **(b)** Damaged and undamaged Ron's Seedling cherry stems. Arrows indicate location of severe earwig cherry damage.

Cherry bunch size in relation to earwig location and cherry damage

To assess the relationship cherry bunch size and cultivar have on the presence of earwigs within bunches and cherry damage, 40 trees were randomly selected from the interplanted RS/ LW block and 20 trees randomly selected from the Sweet Georgia block (Table 4-1). Due to difficulties in finding Lapin cherry blocks with sufficient earwig populations and fruit load during the 2011/12 season, the Lapin cherry bunch, cherry damage and earwig data from the four cardinal limbs of the exclusion experiment were used to generate the data for the Lapin cultivar. Three weeks prior to cherry harvest, cardboard earwig rolls as previously described in the exclusion experiment were tied to the trunk of each tree with garden twine 30 cm from the ground surface. To ensure a broad range of bunch sizes were selected a

maximum of six of each fruit bunch size (1-2, 3-6, 7-12, 13-18, 19-25 and 25+ fruits per bunch) were randomly selected within each tree. All earwig, cherry bunch and damage data were recorded a maximum two days prior to harvest as previously described in the exclusion experiment with the exception of bunch position and limb aspect which were not recorded.

Data Analysis

Data from the exclusion experiment collected to examine the influence limb aspect, bunch position along the limb and earwig trunk trap numbers have on the incidence of cherry fruit and stem damage were analysed using logistic regression with a binary logit link. The relationship between cherry bunch size and earwig numbers found within bunches was also analysed using logistic regression with a log link function for each cultivar. Best regression model fit was assessed using Vuong's closeness tests (Table 4-2). The zero inflated negative binomial distribution (ZINB) was determined to be the best distribution to model the number of earwigs residing within cherry bunches due to the large number of bunches with no earwigs present (AIC = 843). Due to the low number of damaged fruit in the Ron's Seedling blocks regression analysis was not possible and contingency table analysis were performed to assess the impact both limb orientation and bunch position has on fruit and stem damage. Cherry bunch characteristics namely the relationship aspect and bunch position and their interaction have with bunch size, were analysed using a general linear model.

To investigate the relationship between the number of earwigs found within bunches, cherry cultivar and cherry bunch size a generalised linear mixed model using a logit link function and orchard as a random variable was used. Again, Vuong and AIC tests were performed to determine model best fit. A zero inflated Poisson (ZIP) distribution was deemed to be the best distribution to model (Table 4-2) despite ZINB having a stronger AIC (ZIP AIC = 3135; ZINB AIC = 2507). The predictive accuracy of the ZIP models used to examine the relationship between earwig numbers in bunches and cherry bunch sizes were determined using Nash-Sutcliffe efficiency model coefficients (E_f) where E_f ranges from -∞ and 1. An E_f = 1 is deemed an optimal value and an $E_f \leq 0$ indicates an unacceptable model performance and that the observed mean is a better indicator than the predicted value (Moriasi *et al.* 2007). Odds ratios of stem and fruit damage on bunch data between the four varieties were determined using a binomial distribution with earwigs per bunch and cultivar as explanatory variables and tree as a random variable. To compare fruit and stem damage incidence within

varieties Wilcoxon signed ranks tests were performed and Mann-Whitney U tests to compare differences between varieties.

How the level of earwig aggregation may vary across varying cherry bunch sizes and within tree canopies was assessed using the aggregation parameter, theta (θ). Theta values approaching zero indicate a negative binomial (NB) distribution (earwig aggregation) and values approaching infinity indicate a Poisson distribution (random distribution) (Zillio & He 2010). To determine the relationship between bunch size and the level of earwig aggregation, θ estimates were calculated for bunches within each cultivar ranging in size by 12 cherries i.e. bunches containing 2-14 cherries, 3-15 cherries etc. The aggregation behaviour analysis used only bunches where more than one earwig was present. Bootstrapping procedure was used in which the data were re-sampled 100 times using the R sample function.

All data were analysed using SAS version 9.2 with the exception of the non-parametric Mann-Whitney U and Wilcoxon signed ranks tests that were conducted using IBM SPSS Statistics 19 and theta calculations, which were calculated using R (version 2.15.1).

Table 4-2. Vuong closeness test *Z* statistics and preferred model distributions for earwig exclusion and cherry bunch size experiments. ** indicates significant differences < 0.001, * indicates significant differences < 0.05.

		Exclusion experiment		Bunch size experiment	
Model 1	**Model 2**	***Z***	**Preferred model**	***Z***	**Preferred model**
NB	**POI**	7.7*	NB	-5.7*	NB
ZIP	**POI**	9.1*	ZIP	6.6*	ZIP
ZINB	**NB**	20.0*	ZINB	7.7*	ZINB
ZINB	**ZIP**	-2.6*	ZIP	2.7*	ZINB
ZINB	**POI**	1.2	ZINB	7.7**	ZINB
NB	**ZIP**	-1.5	ZIP	-2.6*	ZIP

Results

Cherry bunch sizes within the tree

Cherry bunch sizes varied significantly in RS1, RS2 and Lapin trees with respect to position of the cherry bunch along the limb and the cardinal direction of the limb (Table 4-3). In Lapin where trees were spaced closer together and row orientation was north-west/south-east, larger fruit bunches occurred in limbs on the eastern, western and southern sides of the trees (χ^2 =

16.4, df = 3, $P = 0.001$) with the largest bunches occurring within the outermost third of all limbs ($\chi^2 = 7.7$, df = 2, $P = 0.02$). Conversely, in RS1 and RS2 larger bunches occurred on the eastern limbs of the tree (RS1; $\chi^2 = 18.6$, df = 3, $P < 0.001$ and RS2 ($\chi^2 = 17.8$, df = 3, $P < 0.001$). In RS1, bunch size did not vary along the limb ($\chi^2 = 4.4$, df = 2, $P = 0.11$) however in RS2 larger bunches were observed in the outer third of the eastern and western limbs ($\chi^2 = 25.9$, df = 2, $P < 0.001$).

Table 4-3. Mean bunch size (± SD) of sweet cherries from the four cardinal points and the inner, middle and terminal thirds of the limbs. Cherry number RS1 n = 1314, RS2 n= 1396 and Lapin n = 763.

		Cardinal direction			
Block	**Bunch position**	**North**	**South**	**East**	**West**
RS1	Inner	3.37 (2.11)	3.58 (3.58)	4.13 (2.75)	3.43 (2.43)
	Middle	3.69 (2.59)	4.08 (2.41)	4.58 (3.22)	3.78 (2.95)
	Terminal	3.42 (2.95)	3.60 (3.30)	4.88 (4.61)	3.96 (3.61)
RS2	Inner	5.96 (5.82)	4.70 (3.68)	5.51 (3.76)	5.01 (3.76)
	Middle	5.24 (5.43)	5.05 (5.55)	5.63 (5.11)	6.17 (6.43)
	Terminal	5.40 (7.14)	5.89 (10.36)	9.48 (13.74)	6.05 (9.54)
Lapin	Inner	4.48 (2.79)	5.98 (6.76)	6.89 (5.55)	5.63 (4.40)
	Middle	5.65 (5.35)	7.40 (6.03)	8.85 (10.04)	5.87 (4.85)
	Terminal	7.50 (8.65)	9.44 (11.31)	9.04 (8.29)	10.31 (10.48)

Earwig presence in trees

No significant difference between the two Ron's Seedling blocks with respect to the overall number of earwigs found within the fruit bunches was found ($\chi^2 = 1.8$, df = 1, $P = 0.06$). However, very low earwig numbers were found within the cherry bunches at both sites with a total of 2 earwigs found within all RS1 bunches and 11 earwigs at RS2. Hence, regression modelling of earwig numbers and bunches for RS was not possible. More earwigs were found in RS1 trunk traps than in RS2 ($\chi^2 = 31.0$, df = 1, $P < 0.001$) with low earwig numbers also evident in traps at both locations (mean ± SEM; RS1 2.10 ± 0.04 and RS2 0.55 ± 0.03). Despite low earwig numbers being observed within the cardboard rolls, a visual search of trees showed high numbers of earwigs hiding under tree bark and in cracks within the tree trunks (Quarrell pers. obs.). This hiding in cracks within the tree trunk was observed to occur along a spatial gradient along each row of the block. Similarly, at RS1 numerous earwigs were observed under the cut grass mulch layer under the trees rather than within the cardboard roll trunk traps. These differing hiding sites meant that the low earwig numbers

found in the cardboard earwig rolls in RS1 and RS2 did not accurately represent the size of the earwig populations.

Significant differences were observed between earwig numbers in Lapin and Sweet Georgia trees. More earwigs were found in Sweet Georgia trunk traps (mean ± SEM; Lapin 16.05 ± 2.25; Sweet Georgia 19.75 ± 2.26; $U = 105106$, $Z = 4.1$, $P < 0.001$) and over twice as many earwigs were found within Sweet Georgia cherry bunches (mean ± SEM; Lapin 0.41 ± 0.07; Sweet Georgia 2.06 ± 0.29; $U = 99027$, $Z = 8.6$, $P < 0.001$). Nevertheless numbers in the tree canopy were not high averaging 15.6 earwigs per four limbs or since each Lapin tree possessed an average of 6 limbs, each tree averaged ca. 24.3 (± 2.9) earwigs per tree canopy. Within the interplanted RS/LW block greater earwig numbers were found within the Lewis tree canopies (mean ± SEM, Ron's 0.13 ± 0.05, Lewis 0.37 ± 0.06; $U = 61112$, $Z = -4.1$, $P < 0.001$) but not within the trunk traps where more earwigs were found within the Ron's Seedling traps (mean ± SEM, Ron's 2.9 ± 0.83, Lewis 2.25 ± 0.51; $U = 53403$, $Z = 4.9$, $P < 0.001$). The greatest number of earwigs found aggregating within a cherry bunch was in a Sweet Georgia where 45 earwigs were found within a single bunch of 13 cherries compared to 27 in a Lapin bunch of 15 cherries, 9 earwigs in a Lewis bunch of 46 cherries and 12 earwigs in a Ron's Seedling bunch of a 12 cherries (Figure 4-3). Few other chewing insects i.e. Curculionidae that may have been causal agents for any of the observed damage were observed at any of the sites examined.

In Lapin trees earwigs aggregated more strongly in tree canopies with higher fruit loads ($\theta = 0.49$, $P < 0.001$). More earwigs were found in cherry bunches as size increased for both varieties assessed in the exclusion experiment ($\chi^2 = 214.1$, df = 1, $P < 0.001$) and the four varieties assessed in the bunch size experiment ($\chi^2 = 47.2$, df = 3, $P < 0.001$, Figure 4-2). The Nash-Sutcliffe model efficiency indicates a significant goodness-of-fit in all ZIP regression models developed from bunch experiment data all with $E_f \geq 0.70$.

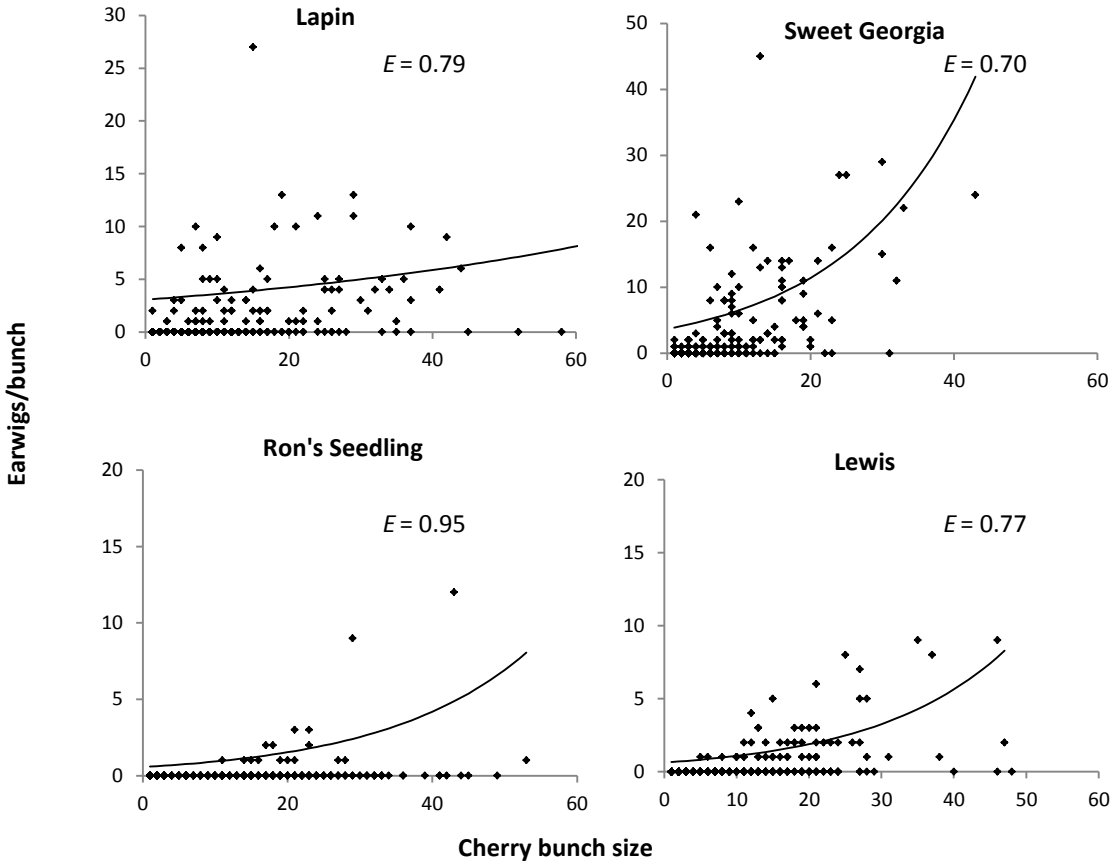

Figure 4-2. Relationship between *Forficula auricularia* aggregation sizes within cherry bunches and cherry bunch size in four varieties of Sweet cherry. Earwigs within Lapin and Sweet Georgia cherries were observed in an organic orchard in the Huon Valley, Tasmania, Lewis and Ron's Seedling cherries were observed in a cherry orchard in Young, NSW.

In Lapin trees earwig presence within fruit bunches did not relate to either the limb's cardinal direction (χ^2 = 5.0, df = 3, P = 0.17) or bunch position (χ^2 = 1.1, df = 2, P = 0.59). However, the interaction of the two was shown to play a role in earwig residence (χ^2 = 14.5, df = 6, P = 0.03, Figure 4-4) where more earwigs were found in the larger, outermost bunches (Table 4-2) at all aspects except on the western side (Figure 4-3).

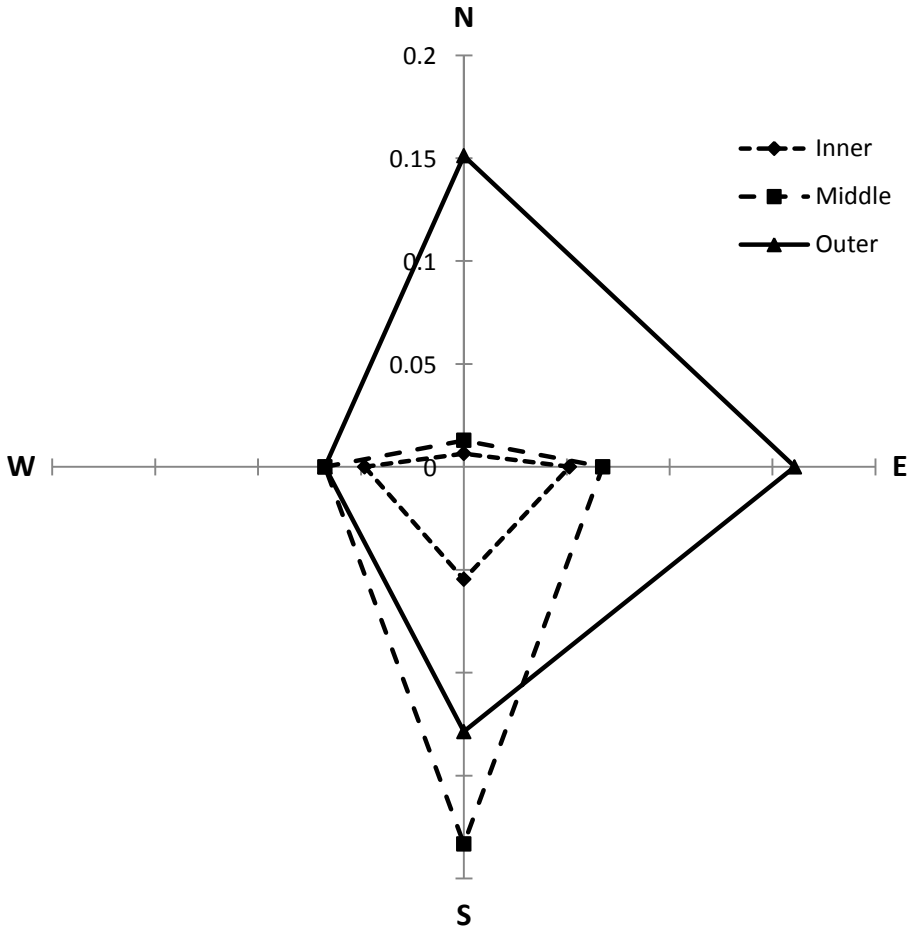

Figure 4-3. Proportion of total *Forficula auricularia* found within cherry bunches in Lapin cherry tree canopies (n = 20) by limb aspect (N, S, E and W) and bunch position along the limb showing a significant preference for bunches in the southern and eastern aspect of the tree and northern most terminal fruit bunches ($P = 0.03$).

The relationship between the aggregation parameter, θ and bunch size indicates earwigs aggregate strongly within cherry bunches, with θ estimates approaching zero at all bunch sizes across all varieties (Figure 4-4a). Similarly, earwigs were not randomly distributed throughout the tree canopy (Figure 4-4b).

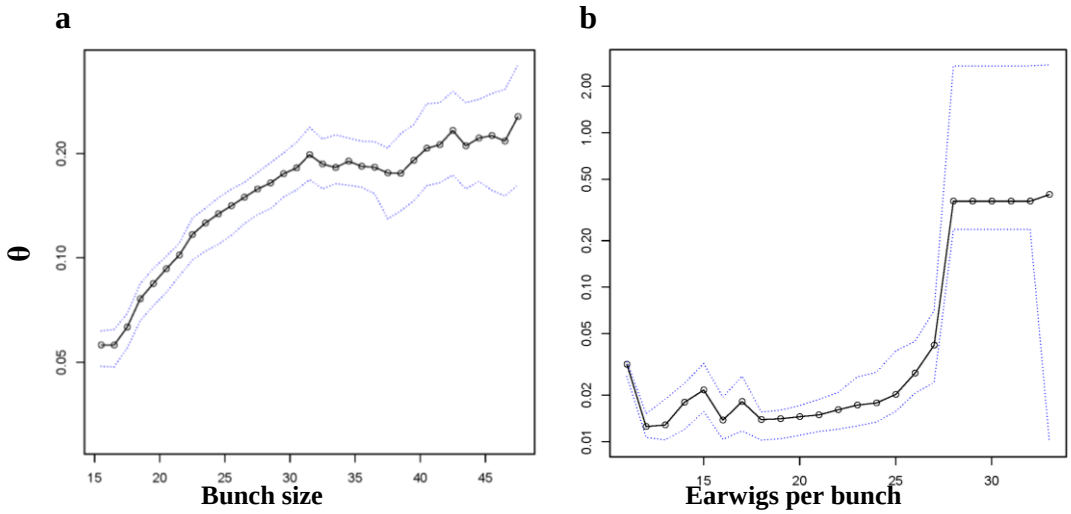

Figure 4-4. *Forficula auricularia* aggregation parameters estimates (θ ± 90% CI) by (a) cherry bunch sizes and (b) earwigs per bunch where > 1 earwigs were present within the bunch. Theta (θ) is the shape parameter of the Negative Binomial distribution. Where distributions approaching zero indicate earwig aggregation (negative binomial distribution) and estimates further from zero ($\theta \rightarrow \infty$) indicate a randomly dispersed earwig population throughout the tree canopy (Poisson distribution)

Earwigs and cherry damage

Damage in earwig absence

Cherry damage was significantly reduced by earwig exclusion. Exclusion bands on the Lapin exclusion limbs significantly reduced the number of earwigs found within the cherry bunches where only one earwig was observed in the exclusion limb bunches (χ^2 = 32.4, df = 4, P < 0.001) thereby significantly reducing the level of fruit damage 9-fold (χ^2 = 59.0, df = 4, P < 0.001) and stem damage 5 fold (χ^2 = 324.3, df = 4, P < 0.001). At both RS sites earwigs were able to circumvent the exclusion bands on some trees. At RS1, a total of 20 earwigs where found in the earwig traps on the exclusion limbs and a total of 48 earwigs within the traps on the exclusion limbs at RS2. Despite this stem damage at both sites was significantly reduced by ca. 2.5-fold (RS1, χ^2 = 16.71, df = 4, P = 0.002; RS2, χ^2 = 24.85, df = 4, P < 0.001). Statistics could not be performed on the Ron's Seedling fruit damage within orchards due to the low number of fruits damaged, however, when the orchards were pooled together there was a significant 3-fold reduction in fruit damage in the exclusion limbs (χ^2 =15.42, df = 4, P = 0.004).

Differences were observed between the two Ron's Seedling orchards with respect to fruit and stem damage (stem: χ^2 = 4.9, df = 1, P = 0.03, fruit: χ^2 = 13.1, df = 1, P < 0.001). In RS1 42.5% (±1.4) stem and 0. 8% (±0.2) fruit damage was observed compared to 37.2% (±1.0)

stem and only 2 individual (< 0.1%) fruit damaged in RS2. Differences in fruit and stem damage between varieties were most evident within the interplanted Ron's Seedling and Lewis block. Ron's Seedling stems were up to 10 times more damaged than Lewis stems (U = 19972, Z = -17.4, P < 0.001, Figure 4-5) and Lewis fruit 1.7% less damaged than Ron's Seedling (U = 61128, Z = 3.2, P = 0.002). Differences in damage were also observed within varieties with Ron's Seedling stem damage on average 11 times higher than Ron's Seedling fruit damage; Sweet Georgia fruit damage two times higher than Sweet Georgia stem damage and Lapin fruit damage two times higher than Lapin stems (Figure 4-5). No significant difference was observed between fruit and stem damage in Lewis trees (Z = 1.5, P = 0.14).

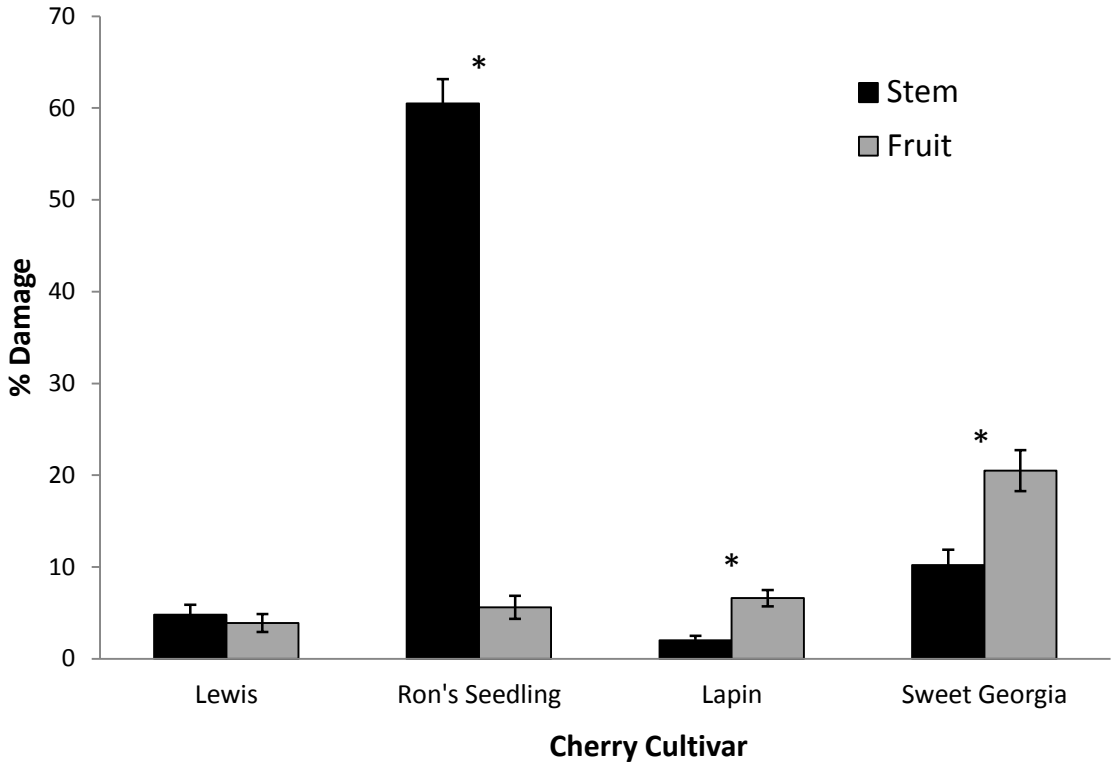

Figure 4-5. Percentage earwig cherry fruit and stem damage (± SE) from four varieties of Sweet cherry observed during the bunch size experiment. Asterisks indicate significant difference between damage types within varieties P < 0.001.

Ron's Seedling stems were 40 times more likely to be damaged when compared to Lewis stems whereas Lewis fruit was five times as likely to be damaged as Ron's Seedling fruit (Table 4-4). Similarly, in the Huon Valley Sweet Georgia fruit were shown to be 4.5 times more likely to be damaged than Lapin fruit and Sweet Georgia stems 5 times more likely to be damaged than Lapin stems (Table 4-4). Overall Sweet Georgia fruit and Ron's Seedling stems were the most likely to be damaged of the four varieties examined.

Table 4-4. Odds ratios (± CI) of stem and fruit damage in four varieties of sweet cherry when *Forficula auricularia* are present within the cherry bunch. Odds ratios indicate the probability of damage occurring when compared to the reference cultivar. Odds ratios below the diagonal are reciprocals of those above. Asterisks indicate significant odds ratios * < 0.05, ** < 0.001.

	Reference cultivar							
	Ron's Seedling		**Lewis**		**Lapin**		**Sweet Georgia**	
Cultivar	Stem	Fruit	Stem	Fruit	Stem	Fruit	Stem	Fruit
Ron's Seedling	-	-	40.48** (21.86, 74.98)	0.45* (0.23, 0.87)	85.11** (45.55, 159.01)	0.24** (0.13, 0.46)	16.17** (8.72, 29.93)	0.05** (0.03, 0.10)
Lewis	0.03** (0.01, 0.05)	2.24* (1.16, 4.42)	-	-	2.10* (1.10, 4.01)	0.54* (0.29, 0.99)	0.40* (0.21, 0.76)	0.12** (0.07, 0.21)
Lapin	0.01** (0.01, 0.02)	4.17** (2.19, 7.92)	0.48* (0.25, 0.91)	1.86* (1.01, 3.43)	-	-	0.19** (0.10, 0.36)	0.22** (0.12, 0.40)
Sweet Georgia	0.06** (0.03, 0.12)	18.81** (9.88, 35.80)	2.50* (1.32, 4.74)	8.41** (4.56, 15.50)	5.27** (2.76, 10.05)	4.52** (2.94, 8.18)	-	-

Significantly more earwig damage was observed on the main limbs than on side branches (χ^2 = 11.2, df = 2, P = 0.004). In Lapin, neither fruit damage nor stem damage were shown to be influenced by bunch position along the limb (Table 4-5) or limb's cardinal direction (Table 4-6) despite recorded differences in cherry bunch size (Table 4-3) and earwig presence (Figure 4-4). In RS1 trees cherry stem damage was not related to either limb aspect (Table 4-6) or bunch position (Table 4-5) but fruit damage was related to limb aspect (Table 4-6) with 1.5% fruit damage on the eastern aspect compared to 0.9% on the southern, 0.4% on the eastern and 0.5% on the northern sides. In RS2 stem damage was not significantly related to bunch position (Table 4-5) but aspect was related with more stems damaged on the western side of the tree compared to the other cardinal points (Table 4-6). The observed gradient in earwig numbers at orchard RS2 correlated with a significant increase in stem damage (χ^2 = 123.7, df = 1, $P < 0.001$) but not fruit damage (χ^2 = 15.0, df = 17, P = 0.60). Low levels of fruit damage observed at RS2 (n = 2) meant analysis could not be performed.

Table 4-5. Percentage fruit and stem damage (± SE) at three bunch positions along tree inner, middle and outer thirds of the limb in two Ron's Seedling and one Lapin cherry block during the 2011/12 season. N/A indicates statistical analysis could not be performed due to an insufficient number of damaged cherries.

		Bunch position on limb							
		Fruit damage (%)				**Stem damage (%)**			
Block	**n**	Inner	Middle	Terminal	*P value*	Inner	Middle	Terminal	*P value*
RS1	5251	0.7 (0.2)	0.7 (0.2)	0.9 (0.2)	0.7	42.7 (1.3)	41.5 (1.1)	43.0 (1.2)	0.6
RS2	8317	0.0 -	0.0 -	0.0 -	N/A	33.9 (1.0)	34.9 (0.9)	40.0 (0.9)	0.1
Lapin	5485	5.6 (0.6)	6.1 (0.6)	7.6 (0.6)	0.06	2.1 (0.4)	1.5 (0.3)	2.4 (0.3)	0.1

Can earwig trunk trap numbers be related to cherry damage?

Unfortunately, no relationship could be ascertained between the total number of earwigs found within the trunk traps at the time of harvest and the level of cherry fruit or stem damage (fruit: $F_{1,3}$ = 0.02, P = 0.90; stem: $F_{1,3}$ = 0.1, P = 0.80) nor the number of male earwigs (fruit: $F_{1,3}$ = 1.6, P = 0.20 stem; $F_{1,3}$ = 0.1, P = 0.74), females (fruit: $F_{1,3}$ = 0.4, P = 0.55; stem: $F_{1,3}$ = 1.2, P = 0.27) juvenile earwigs (fruit: $F_{1,3}$ = 0.3, P = 0.64 stem: $F_{1,3}$ = 1.6, P = 0.43) or the total number of earwigs found within the tree canopies within cherry bunches (χ^2 = 0.6, df = 1, P = 0.45).

Table 4-6. Percentage fruit and stem damage (± SE) in tree limbs at the four cardinal points observed in two Ron's Seedling and one Lapin cherry block during the 2011/12 season. Bold type indicates significant difference at < 0.05. N/A indicates statistical analysis could not be performed due to an insufficient number of damaged cherries.

		Limb aspect									
		Fruit damage (%)					**Stem damage (%)**				
Block	**n**	N	S	E	W	*P value*	N	S	E	W	*P value*
RS1	5251	0.5 (0.2)	0.9 (0.3)	1.3 (0.3)	0.4 (0.2)	**0.01**	49.2 (1.5)	42.1 (1.4)	37.4 (1.3)	41.3 (1.3)	0.8
RS2	8317	0.0 -	0.0 -	0.0 -	0.0 -	N/A	36.7 (1.1)	32.9 (1.1)	34.3 (1.0)	45.0 (1.1)	**0.04**
Lapin	5485	6.9 (0.7)	6.6 (0.7)	5.8 (0.6)	7.1 (0.7)	0.5	1.8 (0.4)	2.6 (0.4)	1.7 (0.3)	1.9 (0.4)	0.05

Discussion

This study demonstrates *F. auricularia* are capable of causing severe economic damage to sweet cherry production. Our results also show clear differences in both damage type and damage frequency between cherry varieties and different orchards. In Ron's Seedling, stem damage ranged from 37% to 60%, which was significantly higher than that for all other varieties examined. Although differences between Ron's Seedling damage levels could be attributed to possible differing earwig population sizes in RS1 and RS2, the differences in fruit and stem damage in the Lewis trees compared to Ron's Seedling trees cannot as these trees were within the one interplanted block (see Table 4-1).

The tendency of certain cherry varieties to form high density fruit bunches toward the outermost third of the limb, may increase a cherry trees' susceptibility to earwig feeding. This is reflected in more earwigs and a greater proportion of damaged fruit and stems, though not significant in all varieties, being typically found within the outer most third of the tree limbs. The limb extremities increased levels of exposure to sunlight may also lead to fruit of a greater maturity when compared to fruit found within the inner parts of the tree canopy. In the southern hemisphere, it is possible that bunches on the cooler, southern and eastern aspects of the tree would provide better daytime residences and the warmer, sunnier northern and western sides of the tree better food resources. Certainly, in block RS2, which contained widely spaced trees we did see a greater level of stem damage on the western side of the tree. Likewise, in the Lapin trees more earwigs were found residing in the cherry bunches on the

cooler, south-eastern side of the trees where stem damage was marginally higher and a trend towards greater fruit damage was also observed on the western facing limbs.

Bunch architecture may also play a critical role in earwig preference of daytime residence and any ensuing damage though this was not explicitly tested. Ron's Seedling appear to produce smaller bunch sizes that have shorter, thicker stems, with a more open, possibly less favoured bunch structure that may be less favoured by earwigs. Whereas varieties such as Sweet Georgia and Lapin, produce large, dense cherry bunches, possibly more favoured by earwigs (S. Quarrell pers. obs.). It is also possible that when earwigs reside in bunches they may daytime feed. Whether *F. auricularia*'s aggregation pheromone plays a role in the formation of large earwig aggregations within bunches is unknown. However, as the theta estimates indicated that the earwigs are not randomly distributed within the tree canopies it is possible that the pheromone does aid in the formation of aggregations within bunches. This could also explain why large earwig numbers were found in some smaller bunches when other neighbouring large bunches contained few to no individuals.

The age of the cherry tree may also be a complicating factor when monitoring earwig populations with traps placed on the tree trunk. Earwigs were frequently observed residing in cracks within the older tree trunks rather than in open fruit bunches or within the cardboard roll traps, which are often used for earwig monitoring in orchards (Moerkens *et al.* 2009; Mueller *et al.* 1988; Nicholas *et al.* 2005). These cracks may also be impregnated with relatively large quantities of aggregation pheromone, which enhances cracks as their chosen daytime residences. If action thresholds can be developed for earwigs in sweet cherry an alternative method of earwig population monitoring will be required, particularly in older trees. These cracks would also create an additional issue when aiming to chemically control earwigs in old trees as insecticide penetration into these spaces is difficult. Trunk trap earwig numbers at the time of harvest were not found to be a useful indicator of cherry damage during this study. However, closer monitoring of earwig population dynamics throughout the cherry growing season may indicate a monitoring time suitable for the development of action (spray) thresholds for IPM cherry production. Furthermore, any model would need to account for cultivar and average fruit bunch size or crop load if it is to provide accurate damage predictions.

The stems of Ron's Seedling were damaged significantly more than the other varieties examined, irrespective of bunch size. This damage, though cosmetic, reduces the marketability of the fruit leading to a reduction is the crops profitability (Bower 1992). The reason for this strong preference for Ron's Seedling stems remains unclear as the resulting damage rarely penetrated the epidermal layer into the stem's vascular tissues, which would contain greater quantities of water, nutrients and carbohydrates. It seems unlikely that the earwigs are aiming to glean greater nutritional uptake from stem consumption although it remains possible that epidermal layer of Ron's Seedling stems is more nutritious compared to the stems of other varieties. The increased susceptibility of Sweet Georgia cherries to earwig damage compared to Lapin cherries also was not well explained by the physical characters we examined. Sweet Georgia was developed from a Lapin sport causing later ripping approximately two weeks after its parent cultivar (James 2011). This mutation appears to have little effect on the physical characteristics of the fruit, bunch size or bunch architecture, but other characters not examined may differ. Indeed, several studies have demonstrated that different varieties show differing sugar, phenolic or organic acid composition (Kelebek & Selli 2011; Liu *et al.* 2011) and that the concentration of these nutrients increase during maturation (Gonçalves *et al.* 2004) when the majority of damage occurs.

This study empirical demonstrates that earwigs can cause severe economic impacts to sweet cherry production and that the nature of this impact significantly differs between varieties examined with Lapin the least prone to earwig damage. The damage type and severity is strongly influenced by numerous factors including bunch size, and bunch position, limb orientation and possibly to a lesser extent, orchard design. However, just why these observed differences occur needs testing if the financial and environmental impacts of earwigs in sweet cherry are to be minimised.

Acknowledgements

We wish to thank the cherry producers Andrew Smith, Scott Coupland and Robert Fitzpatrick who generously provided their time and resources. We also wish to thank Peter Kennedy whose local knowledge of the Young area also helped make this work possible and Nicole Zhang and Mélusine Lefebvre for their assistance with the field data collection. This work has been supported by grant funding from Horticulture Australia Limited (MT 09006).

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Chapter 5 Identification of the putative aggregation pheromone components emitted by the European earwig, *Forficula auricularia*

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Abstract - The European earwig, *Forficula auricularia* is an invasive pest insect found in many temperate regions of the world that is regarded as an urban and agricultural pest causing damage in numerous agricultural crops. Several studies have shown that *F. auricularia* aggregate in large numbers with the use of an aggregation pheromone. However, these studies failed to identify the pheromone component. If isolated this pheromone could be utilised as a monitoring tool or as a "lure and kill" option in areas where earwigs have become problematic. The aim of this study was to isolate and identify the aggregation pheromone of *F. auricularia* using solid-phase microextraction (SPME), solvent washes and thermal desorption of substrates exposed to earwigs. Headspace analysis of aggregating earwigs using SPME yielded numerous compounds including alcohols, aldehydes and fatty acids, none of which induced earwig aggregations. Solvent washes of male, female and juvenile earwigs isolated 51 different branched and unbranched alkanes, alkenes and alkadienes. Substrates exposed to aggregating field populations *in situ* were demonstrated to be attractive to earwigs after less than 24 hours of exposure. Analysis of these substrates using thermal desorption and solvent washes showed that hydrocarbons are the only detectable compounds laid down by earwigs on these surfaces. Significant behavioural responses were observed to synthetic blends of the unsaturated hydrocarbons containing (*Z*)-7-tricosene, (*Z*)-9-tricosene, (*Z*)-7-pentacosene and (*Z*)-9-pentacosene at $\geq$ 25 insect equivalents in field-based bioassays. However, behavioural responses to these blends proved inconsistent particularly later in the field season, possibly due to a missing component within the pheromone blend or plasticity in the pheromones production and subsequent response.

Key Words - *Forficula auricularia*, Aggregation pheromone, Unsaturated hydrocarbons.

INTRODUCTION

The European earwig, *Forficula auricularia* (Dermaptera: Forficulidae) is an invasive insect species, which uses an aggregation pheromone to mediate interactions between conspecifics (Sauphanor 1992; Walker *et al.* 1993; Hehar *et al.* 2008). However, despite several attempts to isolate and identify its aggregation pheromone, its constituent compounds and point of origin remain unknown (Walker *et al.* 1993; Hehar 2007). *F. auricularia*'s defensive compounds 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone, were among the first defensive secretions isolated from an insect (Schildknecht and Weis 1960). Since this study various attempts have been made to isolate and identify the aggregation pheromone (Sauphanor 1992; Walker *et al.* 1993; Hehar 2007). These studies have isolated numerous compounds including *n*-alkanes, methyl-branched alkanes, hydroquinones, benzoquinones, fatty acids, aldehydes, ketones and vanillin from the frass and cuticles of all life stages and both adult sexes. However, the compounds that initiate the aggregative behaviour of *F. auricularia* remain unknown.

The aggregation pheromones' point of origin is disputed by all authors. Sauphanor (1992) concluded the pheromone originated from glands situated in the fore tibia. Walker *et al.* (1993) later demonstrated that solvent washes of the fore tibia were repellent and that male cuticular washes and frass from all members of the population were attractive. It was concluded that the pheromone originates from the male cuticle, which is later consumed post-ecdysis by other members of the population, and is thereby found in the frass of the entire population. However, the frass samples analysed were not collected from the differing sexes and life stages and it therefore remains unclear how this conclusion was reached. Hehar (2007) later verified that aggregation was not mediated by frass but also showed that the pheromone appears to be of cuticular origin, volatile over short distances and produced and responded to by all members of the population.

Walker *et al*. (1993) was the first to demonstrate attraction to a synthetic compound when attraction to both hexadecanoic ($C_{16:0}$) and octadecanoic ($C_{18:0}$) acids at greater than 50 insect equivalents (IE) was observed in the laboratory. As hexadecanoic acid is known to occur in most living organisms (Dijkstra and Segers 2007) this compound may have attracted these omnivores in a food-based response rather than an aggregative behaviour (Walker *et al.* 1993). Hehar (2007) observed attraction to various highly complex synthetic blends

containing up to 30 components including hydroquinones, benzoquinones and fatty acids (including hexadecanoic acid). However, these blends only solicited responses in juveniles when the quinone fractions were removed and no single blend attracted all members of the population as had been demonstrated utilising earwig exposed substrates during the same study (Hehar *et al.* 2008). This may be partially due to quinones being well known defensive compounds in earwigs and therefore may have acted as an alarm pheromone (Schildknecht & Weiss 1960) as flight responses are commonly observed when earwigs emit these defensive secretions, 2-methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone (Walker *et al.* 1993).

One notable omission from the above mentioned aggregation pheromone studies are the numerous alkenes and methyl-branched alkanes identified from juvenile earwig cuticles by Liu (1991). Recently cuticular HCs were shown to be involved in the maternal care behaviours of *F. auricularia* that mediate food provisioning to juveniles (Mas et al. 2009a) and therefore may also play a role in other earwig behaviours including aggregation. Walker *et al*. (1993) reported the presence of some methyl-branched HCs but only briefly mention the presence of a pentacosadiene ($C_{25:2}$) and heptacosadiene ($C_{27:2}$) and did not identify the double-bond positions of these compounds or their subsequent behavioural importance.

Despite differences in attraction between male and female cuticular washes of *F. auricularia*, no chemical differences have been demonstrated with gas-chromatography/ mass-spectrometry (GC-MS) (Sauphanor 1992; Walker *et al*. 1993). This homogeneity seems unlikely given that Walker and Fell (2001) observed males antennal drumming females during courtship behaviour. This behaviour is characteristic of sex determination via cuticular sex pheromones as observed in many species of Blattodea, Diptera and Coleoptera (Blomquist and Vogt 2003; Gemeno and Schal 2004). With recent advances in GC-MS it is possible that this anomaly may be rectified.

This study fully characterises the cuticular HCs of *F. auricularia* and isolates numerous volatile compounds emitted within the earwig's headspace. The compounds found within earwig aggregation sites are also isolated *in situ*, their point of origin determined and the behavioural functions of those compounds are also examined.

METHODS AND MATERIALS

Insect collection.

All earwigs were collected from an organic apple and cherry orchard in the Huon Valley, Tasmania (42˚ 59.755' S, 147˚ 4.328' E). Insects were housed in 330 x 270 mm plastic containers containing field soil and plant debris, fitted with flyscreen tops to aid ventilation, and kept at 20 ˚C, 18L: 6D. Earwigs were fed a mixture of dog food (Natures Gift, protein 12.5%, fat 8%, crude fibre 2% and calcium 1.2%), lettuce and fresh fruit *ad libitum***.** All earwigs analysed were subspecies B, clade B_2 as per Wirth et al. (1998) see Chapter 2.

Laboratory bioassays.

Attraction to substrates previously exposed to earwigs were tested using a bioassay modified from (Hehar *et al.* 2008) between 18th January and 13th March 2012. Ten male, ten female or ten 4th instar juvenile earwigs were placed into 10 cm glass petri dishes lined with filter paper (Whatman® No. 1) for four days. Food and water were provided to earwigs by placing a lid from a 7 mL scintillation vial (PerkinElmer, cat no. 6000179) into the centre of the dish that contained lettuce and water. Food and water was replenished *ad libitum*. Filter papers were stored at – 6 ˚C until required. Control filter papers were initially also exposed to lettuce and water until subsequent GC-MS analysis showed that food exposure did not contaminate the control papers at which time unexposed filter papers were used for control substrates.

Attraction to earwig exposed filter papers was assessed using still-air three chamber olfactometers made from 9 cm plastic petri dishes as described by Takacs and Gries (2001). All experiments began one hour prior to the beginning of an eight hour scotophase. At commencement of each experiment a single male, female or 4th instar juvenile earwig was placed into the centre chamber of the olfactometer under a small polystyrene cup (Solo® P100-0100) and allowed to settle for 15 minutes. The cup was then removed and the insect's position was recorded after 30 minutes, 1 hour and at the beginning of the next photophase.

Chemical Analysis.

Headspace collection. Analysis of earwig headspace volatiles using SPME was performed by placing groups of ten male, female or 3rd and 4th instar juveniles in to 250 mL glass conical flasks. All earwigs were collected between the 8th December 2009 and 10th February 2010. The flasks were subsequently sealed with purpose built glass stoppers with threaded ends

(Brandon Scientific Glassblowing) that enable the attachment of vials caps fitted with PTFE septa (Grace Davison Discovery, cat no. 98735). The earwigs were then left undisturbed for 24 hours without food or water until analysis. Twenty minutes prior to analysis the SPME fibre (Supelco; 75 µm Carboxon/PDMS) were speared into the top of the flask through the septa and allowed to equilibrate for 15 minutes and subsequently analysed by GC-MS.

The SPME fibre was desorbed in the injection port of a Varian 1177 split/splitless injector fitted to a Varian CP 38000 at 280 °C for 5 minutes in splitless mode. The oven temperature was programmed 35 ˚C (4 minute hold) to 270 ˚C (4 minute hold) at 20 ˚C/minute. Carrier gas flow was helium at 1.2 mL/minute using a constant flow mode. The MS was scanned from *m/z* 20 to 350 at 3 scans per second. Six replicates were performed for each adult sex and juvenile group.

HC identification and quantification. Cuticular HCs were identified and quantified by collecting aggregating male (n = 20), female (n = 20) and 4th instar juveniles (n= 20) from the field on the 16th January 2012. Whole bodies were eluted with 900 µL of hexane and *n*-C_{22} standard (2.5 µg and 1.25 µg for adults and juveniles respectively) for one hour. Solvent extractions were then reduced under a gentle flow of nitrogen to 100 µL and transferred into 150 µL Waters inserts (WAT 094171) for GC-MS analysis.

GC-MS analysis of hexane washes was performed with a Varian CP 3800 gas-chromatograph, fitted with a Varian VF5-MS column (30 m, 0.25 mm, 0.25 um film thickness) coupled to either a Varian 1200 triple quadrupole mass spectrometer or a Bruker 300-MS triple quadrupole mass spectrometer in electron ionisation mode using 70 eV electrons. Samples were injected with a Varian CP-8400 autosampler into a Varian 1177 split/splitless injector at 270 °C with a 30:1 split ratio. Oven temperature was programmed from 50 ˚C (2 minute hold) to 150 ˚C at 30 ˚C per minute, then 150 ˚C to 300 ˚C at 8 ˚C/min (1 minute hold). Carrier gas flow was helium at 1.2 mL/minute using a constant flow mode. The MS was scanned from *m/z* 35 to 600 at 3 scans per second.

Methyl-branched hydrocarbons were identified using *n*-alkane standards, mass spectra from a magnetic sector mass spectrometer, mass spectral fragmentation patterns from Doolittle *et al*. (1995) and Kroiss *et al*. (2011) and published retention index data from Carlson et al. (1998) and Katritzky *et al*. (2000). Double bond positions from alkenes and alkadienes were

identified by derivatisation with dimethyl disulfide (DMDS) as per Carlson *et al.* (1989). Derivatised samples were analysed by GC-MS using a method optimised for high boiling compounds (see below). Alkatriene double bond positions were determined using underivatised samples via mass spectral fragmentation patterns as described by Miller (2000) and Conner *et al*. (1980).

Magnetic sector MS. To aid in peak assignment of the methyl-branched HCs representative samples were also analysed on a Hewlett Packard 5890 GC coupled to a Kratos Concept ISQ magnetic sector mass spectrometer, which offered much better sensitivity than the Varian or Bruker benchtop GC-MS. The column was the same as used on both the Varian and Bruker instruments, the carrier gas was helium with a column head pressure of 15 psi, the temperature gradient was 60-150 °C at 30 °C/min, then 150-300 °C at 6 °C/min. Samples were injected in split mode with a split ratio of 10:1. A mass resolution of 1000 was used, and the range from *m/z* 70 to 650 was scanned at 0.8 seconds per decade. Nominal mass data were acquired and processed using Kratos Mach3 software.

'High Boilers' method. Gas chromatography–mass spectrometry (GC-MS) was performed with a Varian CP 3800, fitted with a Varian VF5-MS column (30 m, 0.25 mm, 0.25 um film thickness) coupled to either a Varian 1200 quadrupole mass spectrometer or a Bruker 300-MS TQ mass spectrometer in electron ionisation mode using 70 eV electrons. Injections of 1 µL of derivatised sample were made into a Varian 1177 split/splitless injector at 270 °C with a 20:1 split ratio. Oven temperature was programmed from 60 ˚C to 300 ˚C at 8 ˚C/min (15 minute hold). Carrier gas flow was helium with a 3 mL/minute in constant flow mode. The MS was scanned from *m/z* 20 to 300 for 3 mins then from 35 to 500 at 3 scans per second.

Field-based bioassays.

Trap Age Experiment. Thirty cardboard earwig rolls (8.5 cm x 9 cm) were pre-exposed to a large earwig population in an organic apple orchard for either one week from the 22nd December 2010 or 24 hours on the 13th January 2011. Additional control traps (n = 30) were suspended from apple trellis with Tanglefoot®-coated garden twine to prevent contact with earwigs but allow exposure to the same environment. Aggregation to earwig-exposed and non-exposed (control) traps was then assessed with a paired design by tying a pre-exposed and control trap to 30 different apple trees on the tree trunk 30 cm above ground level in the

same orchard for a further 24 hours, when earwig numbers, adult sex and juvenile life stages were recorded and released at the base of the tree. The 24-hour experiment was then replicated on the 27th January 2011 using corrugated cardboard rolls (9.5 cm x 9.5 cm) lined internally with a 9 cm microfiber filter paper (Whatman, GF/C). After recording the earwig numbers the filter papers were sealed in borosilicate glass vials and immediately chilled and frozen upon return to the laboratory. GC-MS analysis of the filter papers was conducted initially via thermal desorption of 3 x 1 cm^2 of each paper. The filter papers found with the most earwigs were then re-analysed by washing half of each filter paper in 3 x 1 mL of hexane with 1.25 µg of internal standard (*n*-C_{22}). The washes were then dried to 100 µL under a gentle flow of N_2 to concentrate them for analysis.

Volatile chemicals from borosilicate filter papers pre-exposed to earwigs were each analysed by placing a 2.5 cm x 2.5 cm piece of filter paper directly into silicosteel Thermal Desorption System (TDS) tubing with Silane-treated glass wool (Grace Davison, Part no. 4037) placed into either end to prevent sample movement during testing. Each sample (n = 16) was then desorbed in a Markes International Inc. – Unity Thermal Desorption System QUI – 0002. Helium was used as the carrier gas with split desorption conducted at 80 ˚C for 15 minutes for filter paper collections. The TDU trap was an inert sulphur trap (U-T6SUL) held at 25 ˚C. The trap was desorbed at 290 ˚C for 3 minutes. Trap cleaning was performed between each volatile collection (250 ˚C for 5 minutes), new glass wool was utilised for each sample.

Synthetic blend field testing. Earwig attraction to synthetic compounds and cuticular washes were evaluated in paired tests (treatment versus control) with 20 replicates of each treatment in an organic apple and cherry orchard in the Huon Valley, Tasmania. Bioassays were conducted on various dates during the 2011/12 and 2012/13 field seasons (Table 5-3 and 5-4). Cuticular extracts were collected by either immersing the earwigs in hexane for one hour or trickling 3 x 100 µL of hexane down the dorsal and ventral sides of each earwig. The cuticular extracts were then reduced under a gentle flow of N_2 until the required concentration was achieved. All synthetic HCs were sourced from Sigma-Aldrich with the exception of (*Z*)-7-tricosene, which was sourced from Sapphire Bioscience Pty. Ltd. (cat no. 9000313) and (*Z*)-9-tricosene, (*Z*)-7-pentacosene and (*Z*)-9-pentacosene which were synthesised by Dr Jason Smith at the School of Chemistry, University of Tasmania. For the HCs tested each compound was diluted in hexane in concentrations as per Table 5-4. The HCs were then applied (25 µL) to a red rubber septa (Sigma Aldrich, Cat. no. Z565709) and

allowed to air dry for 15 minutes. Control rubber septa were treated with 25 µl of hexane only. Due to their high volatility all headspace volatiles were applied in an undiluted form into gelatine capsules as per Table 5-3. Headspace control treatments received an empty gelatine capsule only. The rubber septa or gelatine capsules were then rolled into corrugated cardboard rolls (8.5 cm x 9 cm), held closed with natural rubber bands.

Traps were placed in orchard rows using a randomised complete block design after 1600 hours on the afternoon of each trial. In each tree, two corrugated cardboard rolls (treatment and control) were attached to the tree trunk 30 cm above the soil surface with garden twine. Each compound was replicated in twenty trees for all field trials. The total number of earwigs, sex and life stage found in each trap was recorded the following morning. However, as the aggregation pheromone is responded to by all members of the population (Sauphanor 1992; Walker *et al.* 1993; Hehar *et al.* 2008) only the total number of earwigs were analysed.

Statistical Analysis

Wilcoxon Sign rank tests were performed on all bioassay data using IBM SPSS Statistics version 19. To determine differences between male, female and juvenile cuticular HC chemistry recursive partitioning was performed. All chemistry data were analysed as a percentage of the total HC composition of each individual to account for differences in body size. Recursive partitioning develops conditional inference trees (Strobl et al. 2009). At each step a null hypothesis of no association is tested between the outcome and the covariates with the processing stopping if the null hypothesis is retained. If the null hypothesis is not retained the covariate with the strongest association is used to split the data into disjoint sets. This process is repeated until no covariate is associated with the data set (Strobl et al. 2009). Recursive partitioning was performed with R version 2.15.1 using the "party" package and the "ctree" function.

RESULTS

Laboratory-based behavioural experiments performed late during the 2011/12 season showed filter papers pre-exposed to males were significantly repellent to females but attractive to juveniles (Table 5-1). However, papers pre-exposed to females were attractive to males, females and juveniles. Whereas papers pre-exposed to juveniles were attractive to males and other juveniles but not to females (Table 5-1).

Table 5-1. Percentage attraction in paired olfactometer testing of *Forficula auricularia* to filter papers exposed to earwigs for a period of four days. Twenty-five replicates were conducted for each bioassay.

	Treatment paper					
	Male		Female		Juvenile	
Insect	% Attract	*P*-value	% Attract	*P*-value	% Attract	*P*-value
Male	56	0.465	82	0.002	24	0.009
Female	15	0.002	72	0.028	35	0.180
Juvenile	75	0.025	76	0.009	72	0.028

Numerous never previously unreported compounds were identified from the earwig headspace utilising SPME including acetone, 2-butanone, 3-pentanone, 2-butanol, 3-Methyl-butanol, 2-methyl-1-propanol. However, only three compounds were consistently isolated from all samples 2-Methyl-1-butanal, 3-Methyl-butanal and 2-Methyl-1-propanal. None of these compounds were isolated from the cuticular solvent washes in either adult sex or juvenile life stage (Figure 5-1).

Hexane washes of earwig cuticles yielded a total of 51 saturated and unsaturated HCs from the cuticles of male, female and juvenile *F. auricularia* including numerous compounds never identified from this species (Figure 5-1, Table 5-2). Quantification of the HC identified from the hexane washes of aggregating males, female and 4th instar juveniles yielded (mean, *n*-C_{22} equivalents ± SD) 8.39 µg (± 5.35), 8.27 µg (± 3.71) and 7.61 µg (± 1.90) per earwig respectively.

Of the 51 HCs identified 41 were methyl-branched alkanes many of which concur with those identified by Liu (1991). However, the dimethyl-alkanes reported by Walker *et al*. (1993) namely, 9,21-dimethyl-nonacosane and 9,23-dimethyl-hentriacontane appear to have been incorrectly identified with the correct identification of these compounds being 9,13-dimethyl-nonacosane and 9,13-dimethyl-hentriacontane. This is due to the fragments *m/z* 140/323 and *m/z* 140/351 reported by Walker *et al*. (1993) corresponding to fragmentation either side of the 9-methyl position for dimethyl-branched nonacosane and dimethyl-branched hentriacontane respectively. However these compounds also possess fragments at *m*/*z* 211, 253 and 295 which are characteristic ions for 9,13-dimethyl-nonacosane (Figure 5-2) and *m*/*z* 211, 253 and 323 which are characteristic ions for 9,13-dimethyl-hentriacontane (Doolittle *et al.* 1995).

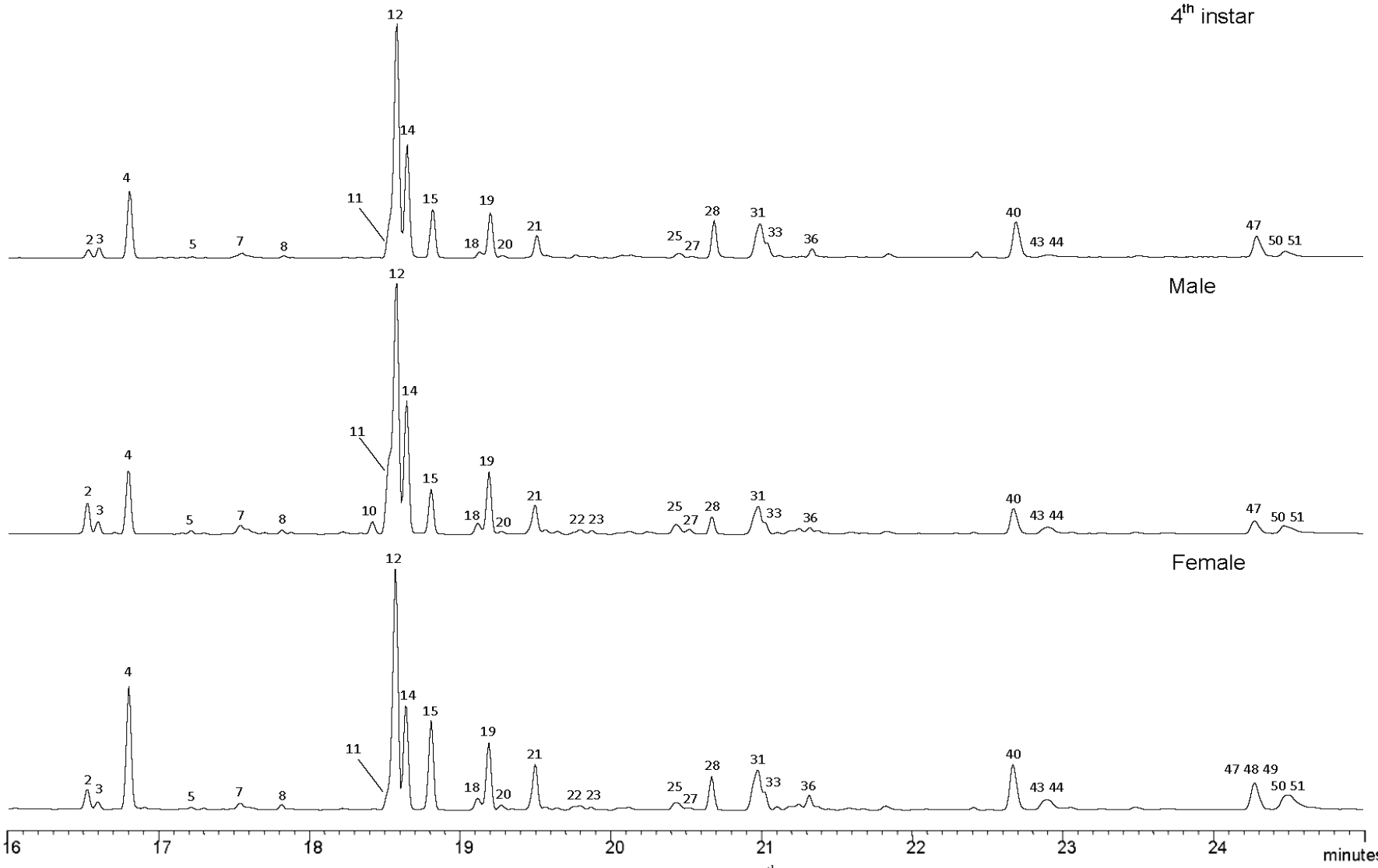

Figure 5-1. Representative gas chromatograms of cuticular hydrocarbon profiles from 4th instar juvenile, adult male and adult female *Forficula auricularia*. Numbers above the peaks refer to compounds listed in Table 5-2.

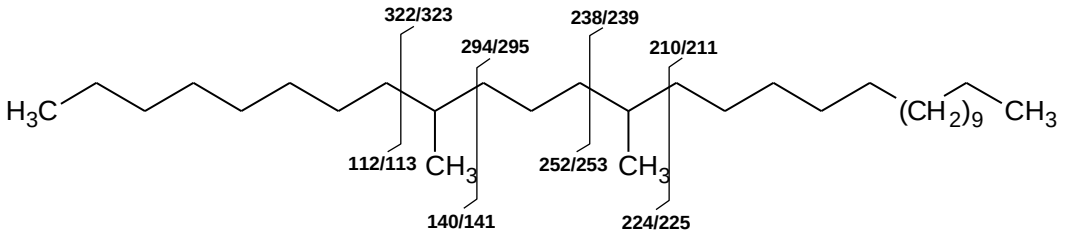

Figure 5-2. Mass-spectral fragmentation pattern of 9,13-dimethylnonacosane

Several alkadienes were identified via DMDS derivatisation coupled with synthetic standards including (*Z*)-9-tricosene, (*Z*)-7-tricosene, (*Z*)-9-pentacosene, (*Z*)-7-pentacosene, (*Z*)-9-heptacosene and (*Z*)-7-heptasosene (Table 5-2). Similarly, DMDS and synthetic standards confirmed the presence of alkadienes (6*Z*,9*Z*)-6,9-pentacosadiene and (6*Z*,9*Z*)-6,9-heptacosadiene. DMDS derivatisation of the alkadienes yielded a complex of six compounds indicated by the scheme observed in Figure 5-3. Two alkatrienes were identified intermittently in the males. These compounds possessed ions at 79, 108, 290 (M-56) and a molecular ion at 346 indicating 3,6,9-pentacosatriene and ions at 79, 108, 318 and a molecular ion at 374 indicative of 3,6,9-heptacosatriene (Miller 2000). As both the alkenes and alkadienes were conformed with a Z configuration with synthetic standards we putatively identify these compounds as (*Z*,*Z*,*Z*)-3,6,9-pentacosatriene and (*Z*,*Z*,*Z*)-3,6,9-heptacosatriene.

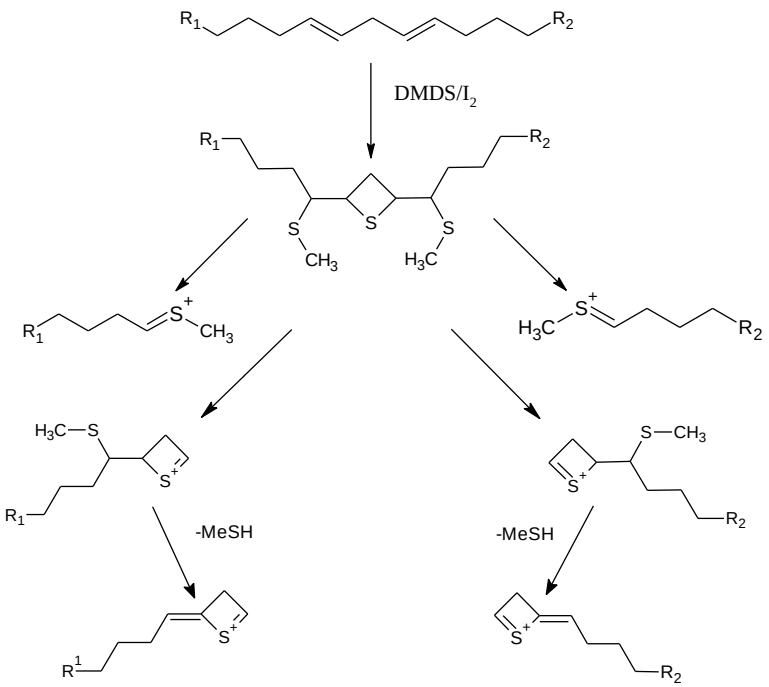

Figure 5-3. Reaction and fragmentation pattern of dimethyl disulfide (DMDS) derivatised methylene interrupted alkadienes.

Table 5-2. Cuticular HC composition (% as *n*-C_{22} equivalents) of aggregating male (n = 20), female (n = 20) and 4th instar juvenile (n = 20) *Forficula auricularia*. Peak numbers denote peaks in Figures 5-2 and 5-5.

				Composition (%, mean ±SD)		
Peak number	**Peak Name**	**RT**	**Identification***	Male	Female	4th instar
1	*n*-C_{21}	14.69	a,b,c,d	0.41 (0.57)	0.29 (0.28)	2.31 (2.02)
2	(*Z*)-9-C_{23}	16.56	a,b,c,d,e	1.73 (1.75)	2.06 (1.76)	5.51 (5.47)
3	(*Z*)-7-C_{23}	16.64	a,b,c,d,e	1.14 (1.12)	1.17 (0.89)	3.32 (1.77)
4	*n*-C_{23}	16.84	a,b,c,d	5.06 (3.55)	4.16 (3.00)	9.41 (3.72)
5	7Me-C_{23}	17.24	b,c	0.04 (0.04)	0.12 (0.10)	0.02 (0.04)
6	5Me-C_{23}	17.34	b,c	0.04 (0.05)	0.09 (0.10)	0.01 (0.04)
7	3Me-C_{23}	17.57	b,c	0.23 (0.17)	0.45 (0.37)	0.34 (0.17)
8	n-C_{24}	17.86	a,b,c,d	0.39 (0.24)	0.40 (0.26)	0.23 (0.11)
9	3,7-diMe-C_{23}	17.92	b,c	0.04 (0.04)	0.26 (0.29)	0.18 (0.19)
10	6,9-C_{25}^	18.48	a,b,c,d,e	0.20 (0.18)	0.22 (0.20)	0.04 (0.11)
11	(*Z*,*Z*)-6,9-C_{25}	18.56	a,b,c,d,e	1.15 (0.81)	1.36 (0.99)	2.03 (0.99)
12	(*Z*)-9-C_{25}	18.59	a,b,c,d,e	8.52 (5.83)	11.66 (10.07)	33.06 (14.15)
13	3,6,9-C_{25}^	18.61	b,c	0.04 (0.06)	-	-
14	(*Z*)-7-C_{25}	18.67	a,b,c,d,e	4.19 (3.52)	5.66 (4.12)	12.81 (5.96)
15	*n*-C_{25}	18.84	a,b,c,d	10.48 (5.63)	9.02 (5.17)	4.93 (3.34)
16	13Me-C_{25}	19.15	b,c	0.32 (0.20)	0.46 (0.30)	0.22 (0.18)
17	11Me-C_{25}	19.16	b,c	0.31 (0.21)	0.63 (0.51)	0.27 (0.20)
18	9Me-C_{25}	19.16	b,c	0.11 (0.08)	0.24 (0.21)	0.13 (0.14)
19	7Me-C_{25}	19.22	b,c	1.85 (1.37)	3.13 (2.28)	2.05 (1.14)
20	5Me-C_{25}	19.26	b,c	0.24 (0.18)	0.41 (0.30)	0.36 (0.35)
21	3Me-C_{25}	19.53	b,c	2.39 (1.51)	3.30 (2.04)	3.03 (1.44)
22	*n*- C_{26}	19.79	a,b,c,d	0.55 (0.31)	0.50 (0.29)	0.15 (0.13)
23	3,7diMe-C_{25}	19.84	b,c	0.07 (0.07)	0.29 (0.22)	0.06 (0.08)
24	6,9-C_{27}	20.45	a,b,c,d,e	0.57 (0.51)	0.34 (0.28)	0.13 (0.11)
25	(*Z*)-9-C_{27}	20.48	a,b,c,d,e	0.50 (0.52)	0.53 (0.50)	0.32 (0.30)
26	3,6,9-C_{27}^	20.54	b,c,d	0.05 (0.06)	-	-
27	(*Z*)-7-C_{27}	20.56	a,b,c,d,e	0.40 (0.51)	0.90 (1.96)	0.11 (0.15)
28	*n*-C_{27}	20.70	a,b,c,d	8.11 (4.66)	5.84 (3.50)	3.01 (2.57)
29	13Me-, 15Me-C_{27}	20.97	b,c	2.13 (1.20)	1.88 (1.05)	0.74 (0.51)
30	11Me-C_{27}	20.98	b,c	2.42 (1.31)	2.62 (1.39)	1.27 (0.83)
31	9Me-C_{27}	21.01	b,c	4.66 (2.56)	4.66 (2.47)	1.75 (1.09)
32	7Me-C_{27}	21.05	b,c	0.23 (0.20)	0.33 (0.32)	0.21 (0.13)
33	5Me-C_{27}	21.10	b,c	0.46 (0.26)	0.37 (0.22)	0.26 (0.20)
34	11,15-diMe-C_{27}	21.20	b,c	0.28 (0.21)	0.29 (0.23)	0.07 (0.16)
35	9,13-diMe-C_{27}	21.26	b,c	0.48 (0.34)	0.68 (0.50)	0.08 (0.09)
36	3Me-C_{27}	21.35	b,c	1.90 (1.07)	1.74 (1.00)	1.36 (0.84)
37	*n*-C_{29}	22.43	a,b,c,d	1.41 (1.04)	0.85 (0.60)	0.36 (0.35)
38	15Me-C_{29}	22.67	b,c	1.95 (1.13)	1.63 (1.07)	0.26 (0.39)
39	13Me-C_{29}	22.67	b,c	2.55 (1.52)	2.08 (1.29)	0.72 (0.53)
40	11Me-C_{29}	22.69	b,c	7.10 (3.85)	5.28 (3.00)	2.45 (1.56)
41	9Me-C_{29}	22.62	b,c	3.98 (2.19)	3.54 (2.03)	1.26 (0.82)
42	7Me-C_{29}	22.70	b,c	1.49 (1.49)	0.68 (0.48)	0.50 (0.39)
43	11,15-diMe-C_{29}	22.79	b,c	1.17 (0.77)	1.07 (0.77)	0.29 (0.63)
44	9,13-diMe-C_{29}	22.82	b,c	0.94 (0.64)	1.43 (1.10)	0.18 (0.35)
45	3Me-C_{29}	22.93	b,c	0.30 (0.19)	0.26 (0.17)	0.14 (0.13)
46	15Me-C_{31}	24.16	b,c	1.56 (0.98)	1.38 (0.91)	0.39 (0.23)
47	13Me-C_{31}	24.17	b,c	3.10 (1.87)	2.56 (1.68)	0.68 (0.45)

48	11Me-C_{31}	24.19	b,c	4.69 (2.64)	4.35 (2.54)	1.69 (0.98)
49	9Me-C_{31}	24.22	b,c	2.77 (1.63)	2.03 (1.24)	0.79 (0.51)
50	11,15-diMe-C_{31}	24.45	b,c	3.37 (2.70)	4.41 (3.87)	0.35 (0.38)
51	9,13-diMe-C_{31}	24.46	b,c	2.00 (1.69)	2.40 (2.18)	0.12 (0.22)

[*]Identification of compounds based on; [a] synthetic standards, [b] MS fragment interpretation, [c] Published Kovats Indices, [d] Published MS spectra, [e] DMDS derivatisation, [^] Stereochemistry not determined with synthetic standards

The conditional inference regression tree highlights cuticular HC differences between adults and juveniles and between adult between males and adult females (Figure 5-4). Node one indicates that 4th instar juveniles can be differentiated from adults in that > 21.48% of their total cuticular profiles are (*Z*)-9-pentacosene ((*Z*)-9-C_{25}, Table 5-2, Figure 5-1, peak 12). Nodes 2 and 3 indicate adult male and female profiles differ in their levels of 3,7-dimethyl-pentacosane (peak 23) and 3,7-dimethyl-tricosane (peak 9) where terminal node 6 indicates that the majority of female profiles were found to possess > 0.19% 3,7-dimethyl-pentacosane and terminal node 4 indicates adult male profiles possess < 0.12% 3,7-dimethyl-tricosane.

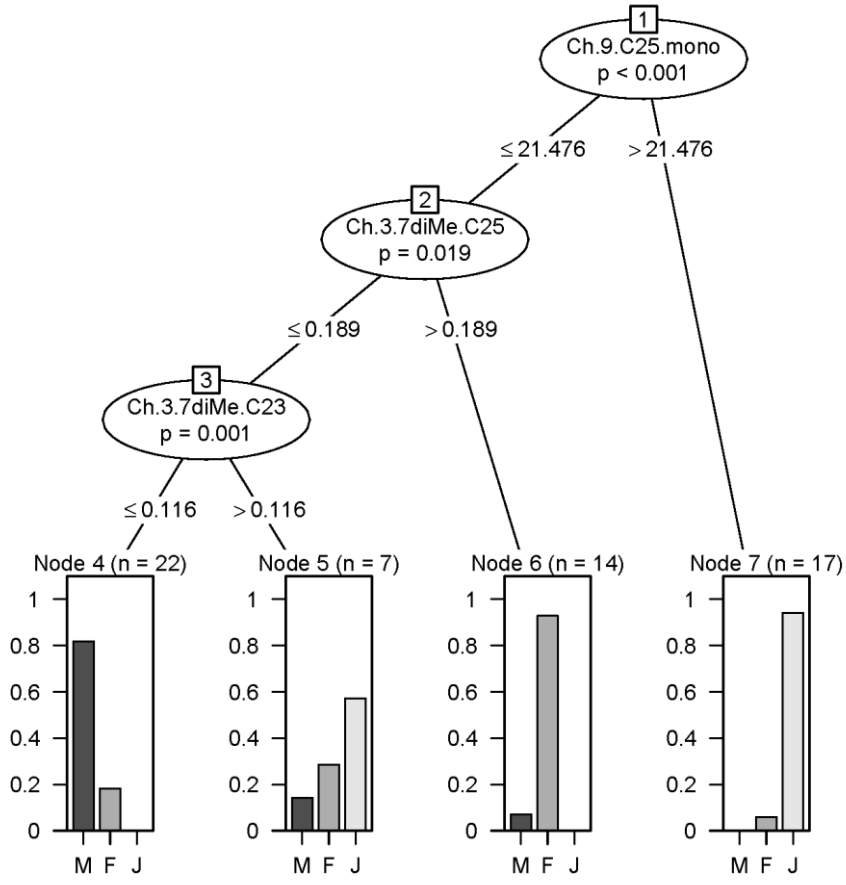

Figure 5-4. Recursive partitioning decision tree indicating cuticular HC differences between field collected male, female and juvenile *Forficula auricularia*. All earwigs were collected on the 16th January 2012. The number of individuals within each terminal node is denoted by the n-value above each bar chart. The bar charts signify the proportion of males (M), females (F) and 4th instar juveniles (J) within each terminal node.

Trap age experiment

More earwigs were observed in traps previously occupied by earwigs for one week than in traps that had not been exposed to earwigs (Figure 5-5; Wilcoxon Sign Rank: $Z = -3.494$ $P < 0.001$). Furthermore, a significant correlation was observed between the number of earwigs found in the traps at the end of the trap pre-treatment phase and the number of earwigs found after the 24 hour experimental phase (Spearman's rho = 0.422, $P = 0.020$). When replicated with a 24 hour pre-treatment more earwigs were observed in the previously exposed traps (Wilcoxon Sign Rank: $Z = -3.530$, $P < 0.001$). However, a correlation between the number of earwigs observed after the 24 hour pre-treatment and after the experimental period was not observed (Spearman's rho = 0.28, $P = 0.883$).

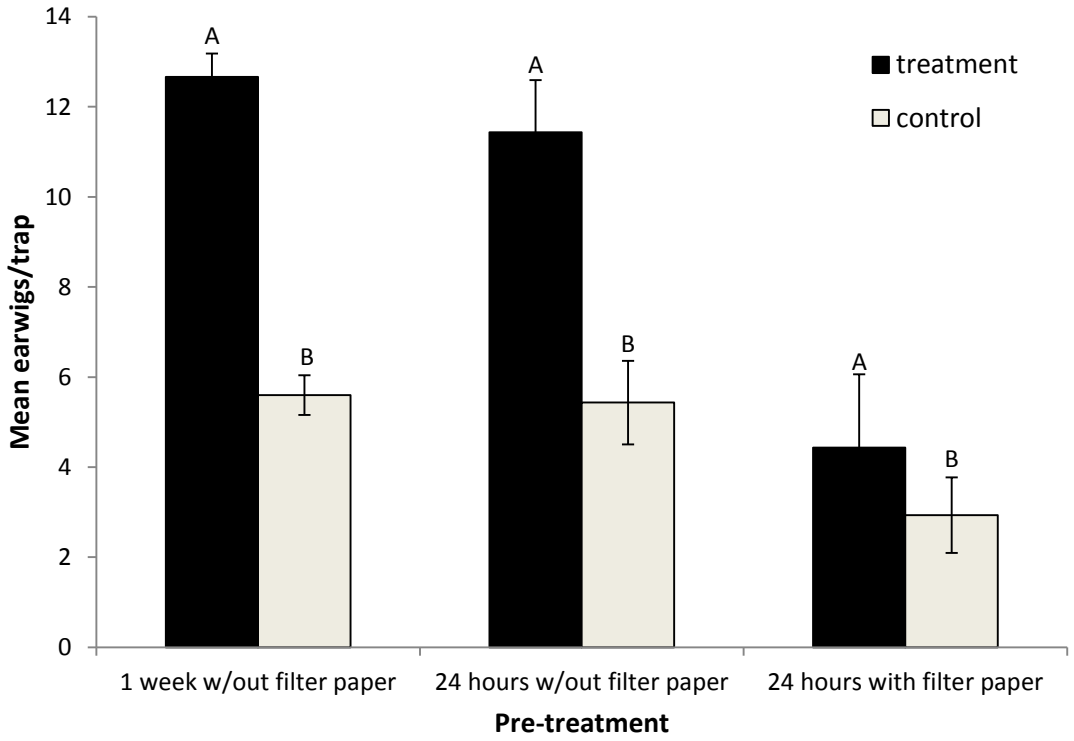

Figure 5-5. Mean (± SEM) earwigs per trap found during the trap age experiment. Letters indicate significant differences within experiments ($P < 0.05$). The one week experiment was conducted on the 22nd December 2010 and the 24 hours on the 13th January 2011 and the 27th January 2011 respectively.

When borosilicate glass filter papers were incorporated in to the earwig traps and exposed to earwigs for 24 hours, again, more earwigs were observed in the traps pre-exposed to earwigs (Wilcoxon Sign Rank: $Z = -2.218$ $P = 0.027$). Chemical analysis of the filter papers using either thermal desorption or hexane washing showed the presence of 37 of the 51 cuticular

HCs previously isolated from earwig cuticle and some solvent residues (Figure 5-6, Table 5-2). Pre-treated traps contained more HC (Wilcoxon Sign Rank Z = -2.380, P = 0.017) than the untreated control traps (mean ± SD; treatment traps 0.29 µg ±0.15; control traps 0.09 µg ± 0.03).

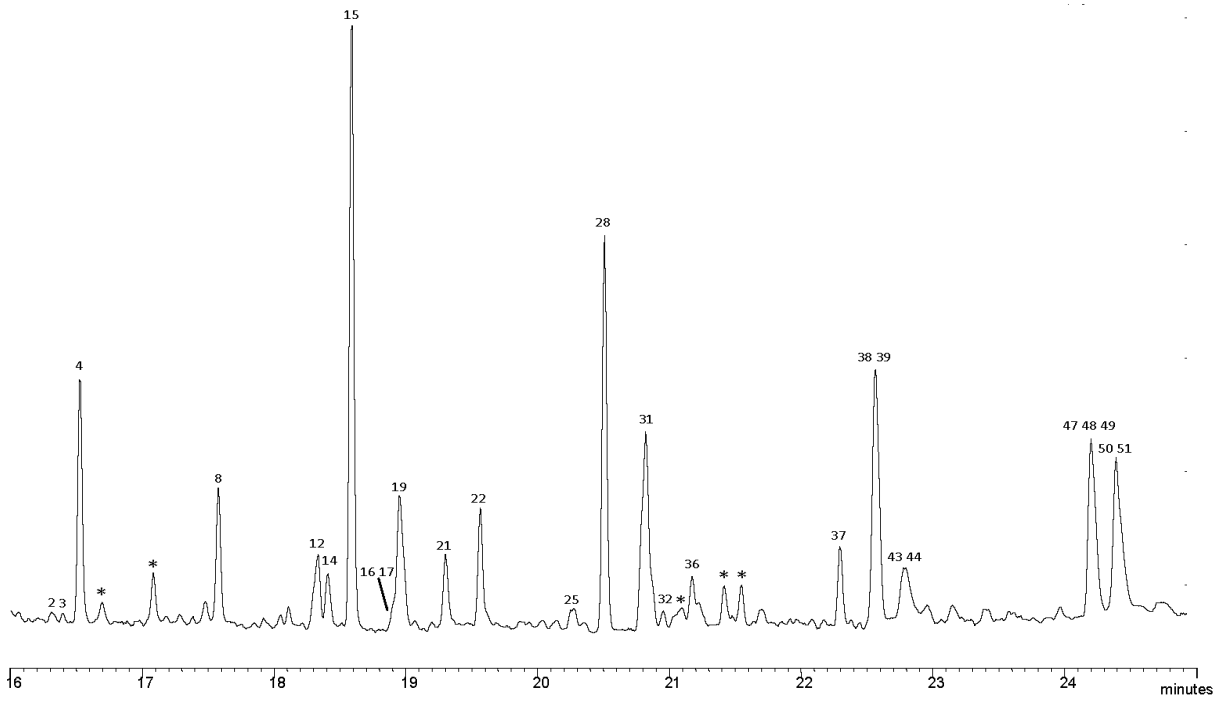

Figure 5-6. Representative gas chromatogram of a filter paper pre-exposed to *Forficula auricularia* for 24 hours used during the trap age experiment. Numbers above the peaks refer to compounds listed in Table 5-2. Asterisks indicate artefact peaks.

Field-based bioassays

Field-based behavioural experiments examining earwig attraction to synthetic lures containing aldehydes isolated from the earwig headspace did not illicit significant earwig attraction nor repellency at either concentration or when tested as single components or as a blend (Table 5-3).

Table 5-3. Mean (± SEM) earwig (total male, female and juveniles) treatment effect (TE; treatment – hexane control) to headspace volatiles after a 12 hour period in field based experiments. Positive numbers indicate attraction. Negative numbers indicate repellency. Compounds were tested within apple trees (n = 20) in a paired design against hexane controls tested on either the 16th January 2011 (0.2 mg) or the 27th January 2011 (0.05 mg).

	Concentration					
	0.2 mg			**0.05 mg**		
Compound	**TE**	***Z***	***P*-value**	**TE**	***Z***	***P*-value**
2-Me-1-propanal	-0.15 (0.68)	-0.918	0.359	0.05 (0.68)	-0.130	0.896
2-Me-1-butanal	0.10 (0.56)	-0.064	0.949	-0.20 (0.72)	-0.088	0.930
3-Me-1-butanal	-0.65 (0.94)	-0.190	0.849	-0.85 (0.65)	-1.356	0.175
Aldehyde blend[a]	-0.35 (0.65)	-1.124	0.261	-0.60 (0.66)	-0.341	0.733
Mean earwigs/trap	3.43 (0.32)			2.66 (0.23)		

[a] aldehyde blend ratio 20:40:40

Mean trap catch numbers varied greatly between field tests (Table 5-4) from 1.28 (± 0.17) per trap on the 19th January to 21.15 (± 1.62) on the 22nd December 2012 with 109 earwigs recorded in a single trap on the 7th December 2012. With the exception of the male wash on the 6th January 2012, which was significantly repellent to other males (Appendix 1; Wilcoxon sign rank; Z = -2.271, P = 0.023) cuticular washes from either males or females did not induce earwig aggregations in field tests (Appendix 1). Significantly more males were observed in traps containing the four alkane blend on the 6th January (Wilcoxon sign rank; Z = -2.271, P = 0.023) where (mean ± SEM) 0.82 (± 0.19) males were observed in the treatment traps compared to 0.25 (± 0.10) males in the controls. None of the alkenes when tested individually elicited a behavioural responses with both (*Z*)-7-C_{23} and (*Z*)-7-C_{25} being slightly though not significantly repellent. However, the two component synthetic alkene blend consisting of (*Z*)-9-C_{23} and (*Z*)-9-C_{25} was attractive to both adult sexes and 4th instar juveniles, which represented the majority of the juvenile life-stages (4th instars 10.5%, 3rd instars 0.5% of the total earwig population) in the field at that point in time (Figure 5-7, Table 5-4; Wilcoxon sign rank; trap total Z = -3.086, P = 0.002; males Z = -3.078, P = 0.002 females Z = -2.313, P = 0.021, 4th instars Z = -2.332, P = 0.020), where (mean ± SEM) 7.42 (± 1.17) earwigs were caught in the treatment traps compared to 3.11 (± 0.69) in the control traps.

On several occasions the four component synthetic alkene blend consisting of (*Z*)-9-C_{23}, (*Z*)-7-C_{23} (*Z*)-9-C_{25} and (*Z*)-7-C_{25} at 25 insect equivalents (IE) (0.05 mg), 50 IE (0.1 mg) and 100 IE (0.2 mg) elicited significant increases in the total trap catches (Table 5-4). On the 2nd

December 2012 (Wilcoxon sign rank; Z = -2.763, P = 0.006) at a concentration of 0.05 mg (mean ± SEM; treatment 2.45 ± 0.47, control 1.25 ± 0.39) on the 6th January 2012 and 7th December 2012 at a concentration of 0.1 mg (Wilcoxon sign rank; Z = -3.421, P < 0.001 and Z = -3.421, P < 0.001 respectively) where 26.30 ± 5.56 earwigs were found in the treatment traps and 15.35 ± 4.68 earwigs in the controls. On the 7th December 2012, at a concentration of 0.2 mg attraction was also observed (Wilcoxon sign rank; Z = -3.264, P < 0.001) where 25.05 ± 3.10 earwigs were observed in the treatment traps compared to 15.95 ± 2.23 earwigs in the control traps. On the 19th January, attraction was observed but by 4th instars only (Table 5-4; Wilcoxon sign rank; Z = -2.000, P = 0.046) when they represented ca. 34% of the population (Figure 5-7). Early season 3rd and 4th instar juveniles demonstrated the most consistent results to the 4 component blends where attraction was observed and on the 2nd December (4th instars Z = -2.249, P = 0.025; 3rd instars, Z = -2.541, P = 0.011) and the 7th December (4th instars Z = -3.194, P < 0.001; 3rd instars, Z = -3.421, P < 0.001) when these life-stages dominated the population (Figure 5-7). However, when replicated on the 22nd December when the juvenile life-stages still comprised the majority of the population no significant responses were observed (P > 0.05, Appendix 1). No attraction was observed to any blend, at any concentration on the 23rd February 2012, 22nd December 2012 or on the 6th February 2013 (Table 5-4).

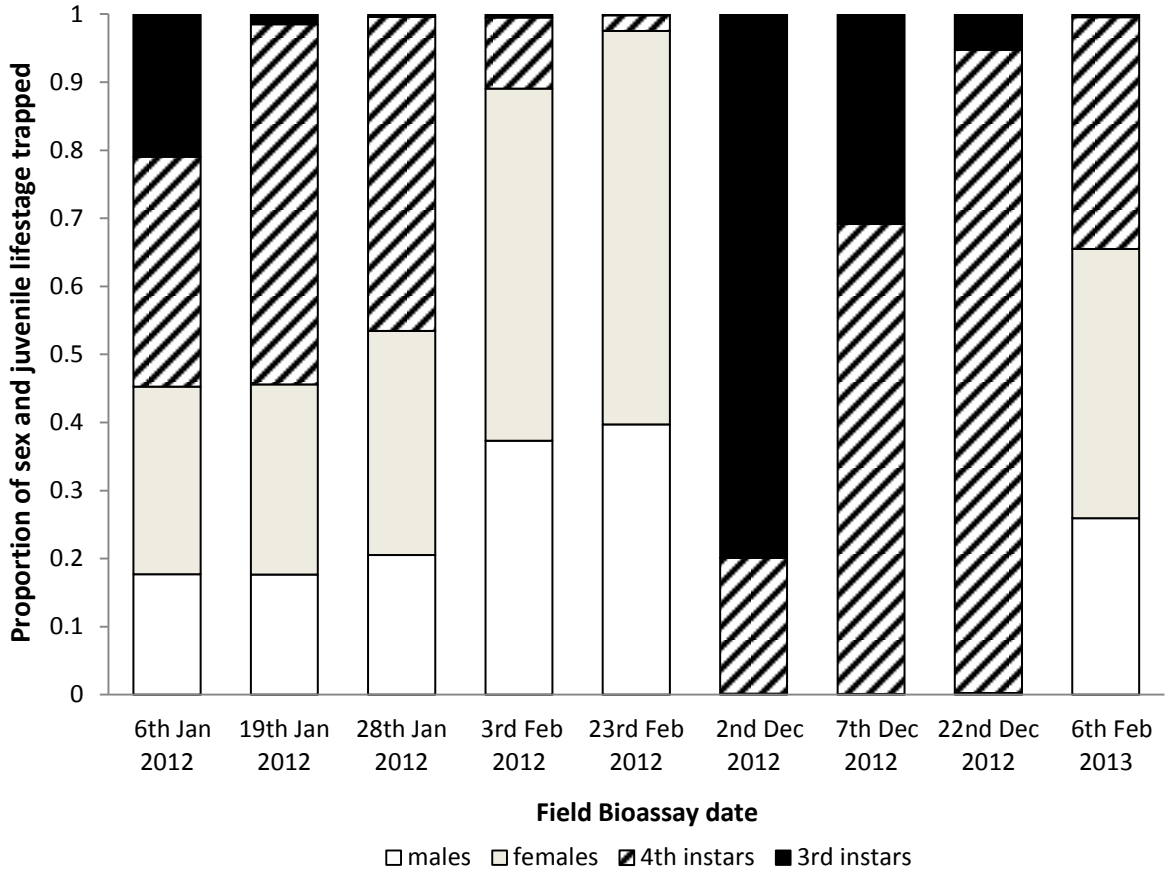

Figure 5-7. Proportion of *Forficula auricularia* males, females, 4th instar juveniles and 3rd instar juveniles trapped during synthetic HC pheromone field testing between the 6th January 2012 and the 6th Febuary 2013.

Table 5-4. Mean (± SEM) *Forficula auricularia* per trap per tree (male, female and juveniles) and mean (±SEM) treatment effect (treatment – hexane control) to hydrocarbons in field based experiments. Positive numbers indicate attraction. Negative numbers indicate repellency. Compounds were tested within apple and cherry trees (n = 20) in a paired design against hexane controls. Bold type indicates significant difference Wilcoxon sign rank < 0.05.

		Field experiment date								
Compound	**Concentration**	**6th Jan 2012**	**19th Jan**	**28th Jan**	**3rd Feb**	**23rd Feb**	**2nd Dec**	**7th Dec**	**22nd Dec**	**6th Feb 2013**
Mean earwigs/trap		1.91 (0.23)	1.28 (0.17)	3.27 (0.36)	5.29 (0.67)	2.85 (0.29)	17.85 (1.91)	20.32 (1.73)	21.15 (1.62)	3.01 (0.39)
Male wash	10 IE[#]	-0.15 (1.56)[*]	0.30 (0.26)[^]							
Female wash	10 IE[#]		0.40 (0.76)[^]							
***n*-alkane blend[a]**	0.1mg	0.25 (0.86)								
HC blend 2[b]	0.1mg	0.25 (2.06)								
(*Z*)-9-C_{23}	0.1mg			0.84 (3.17)						
(*Z*)-7-C_{23}	0.1mg			-1.50 (5.36)						
(*Z*)-9-C_{25}	0.1mg			1.05 (3.89)						
(*Z*)-7-C_{25}	0.1mg			-0.65 (4.78)						
alkene blend 3[c]	0.05mg				1.10 (1.10)		**11.35 (3.97)**	-2.60 (2.18)	-0.55 (3.76)	-0.35 (0.51)
alkene blend 3[c]	0.1mg	**1.20 (1.33)**	0.60 (1.54)	1.95 (8.75)	-1.61 (1.00)			**10.95 (2.16)**	-2.35 (2.79)	-0.05 (0.45)
alkene blend 3[c]	0.2mg		0.50 (1.28)	0.25 (6.31)				**9.10 (2.35)**	-3.35 (3.22)	-0.70 (1.28)
alkene blend 4[d]	0.05mg					1.10 (1.45)	2.25 (1.73)			
alkene blend 4[d]	0.1mg				**4.32 (1.03)**	-0.05 (2.80)		3.1 (2.28)	4.45 (3.38)	-0.05 (0.64)
alkene blend 4[d]	0.2mg					-0.70 (2.80)				
alkene blend 5[e]	0.1mg					1.74 (2.04)				
alkene blend 5[e]	0.2mg					0.30 (1.50)				

[#]IE = Insect Equivalents, [*] 1 hour hexane extraction, [^] 3 x 100 µL cuticular hexane wash
[a] *n*-alkane blend; *n*-C_{21} : *n*-C_{23} : *n*-C_{25}; Blend ratio: 40:85:70
[b] Seven component blend *n*- C_{21} ; (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : *n*- C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25} : *n*- C_{25}; Blend ratio: 40: 70:20:85:80:15:70
[c] Four component blend (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25}; Blend ratio: 60:15:100:25
[d] Two component blend (*Z*)-9-C_{23} : (*Z*)-9-C_{25}; Blend ratio: 30:70
[e] Two component blend (*Z*)-7-C_{23} : (*Z*)-7-C_{25}; Blend ratio: 30:70

DISCUSSION

Although *F. auricularia* is commonly regarded as an odorous species (Fulton 1924; Kehrli et al. 2012) this study shows that the pungent, highly volatile compounds emitted by this species are not those utilised to initiate earwig aggregation. Indeed, our results suggest that it is cuticular HCs that appear to mediate the formation of *F. auricularia* aggregations. Previously, Dermapteran cuticular HCs had been largely attributed to waterproofing and protection from predators either by the dissolution of defensive secretions or by providing earwigs with a slippery cuticle making capture difficult (Walker *et al.* 1993). However, this class of compounds has more recently been implicated in regulating maternal care in this insect (Mas et al. 2009b; Mas and Kölliker 2011). It would therefore appear that despite these long chain HCs being generally regarded as non-volatile (Ozaki and Wada-Katsumata 2010) that their volatility is sufficient to be detected at least over short distances and initiate earwig aggregations (Hehar *et al.* 2008).

Our results also show that these compounds are laid down on substrates, which are in turn attractive in both the laboratory and field. However, the observed attraction to the synthetic alkene blends consisting of (*Z*)-7-tricosene, (*Z*)-9-tricosene, (*Z*)-7-pentacosene and (*Z*)-9-pentacosene in field-based behavioural tests was variable. This failure to consistently replicate field results may be linked to two possible factors; the lifecycle of *F. auricularia* whose trap catches are commonly known to decline from mid-summer (Quarrell 2008; Moerkens *et al.* 2009) or the synthetic blends tested being incomplete, as the alkadiene (*Z,Z*)-6,9-pentacosadiene, was unable to be tested due to issues with acquiring this compound in sufficient purity. This compound may prove important in improving the ability of the pheromone alkene blend to attract and initiate earwig aggregation behaviours as minor components are known to be important with respect to behavioural activity in several other insect species (Walker et al. 2009; Ferveur and Cobb 2010).

The laboratory bioassays conducted during this study point toward the possibility that the production and/or response of the aggregation pheromone used by *F. auricularia* may vary throughout the insect's activity season. This is because our results differed to previous studies which found in laboratory-based colonies that all members of the population respond to substrates exposed to other members, irrelevant of the sex or life-stage (Sauphanor 1992; Walker *et al.* 1993; Hehar *et al.* 2008). Our laboratory bioassays were conducted later in the

earwig season using field collected insects, when sexual status of the adults may have impacted on our results. Indeed, juvenile hormone is known to control oogenesis and maternal care in earwigs (Rankin *et al.* 1997) and therefore may also regulate earwig pheromone production as has been observed in other long-lived insects (Barth 1965; Schal *et al.* 2003)

Walker *et al*. (1993) hypothesised that a number of functions may be served by earwig aggregations including mate finding in adults, predator defence and increased juvenile growth and development. Our results would appear to concur with this hypothesis where differing behaviours were observed dependant on the type of pre-exposed substrate provided and the sex or life-stage of the test subject. In our experiments, females were not attracted to juveniles and were significantly repelled by male exposed substrates. The females used in these experiments were neither displaying nesting behaviours, nor gravid and therefore would not necessarily be expected to display maternal care behaviours or respond to unsolicited attention from males. Similarly, males may seek to avoid other males to limit mate competition as was also observed in both laboratory and field experiments when attraction to cuticular washes from males and male earwig exposed substrates was assessed. However, if juveniles are aggregating so as to enhance their survival and growth it would be expected they be attracted to all members of the population as was also observed in this study. These bioassays need further replication with recently moulted adults to determine whether adult responses to substrates exposed to differing adult sexes and juveniles do change as adults approach sexual maturity.

In previous studies, Hehar (2007) showed male body washes to be repellent; however, Walker et al (1993) found male cuticles to be attractive but female cuticular washes to be slightly though not significantly repellent. Our results showed that neither male nor female cuticular washes were attractive. The reason for this remains unclear as chemical analysis of cuticular washes and earwig exposed substrates showed that HCs were the only compounds laid down on substrates in field-based aggregations. However, the ratio of compounds does appear to differ between the cuticles and the substrates exposed to them. As mixed aggregations occur in the field it may be that the ratios with solvent washes vary enough from those laid down naturally to prevent behavioural responses from occurring.

One other potential complicating factor in the laboratory bioassays used during this and other earwig aggregation studies was that the earwigs were allowed to freely explore both the treatment and control traps to make a choice as to where they wished to reside during the following photophase. Although this method enables the earwigs to reject their initial choice of daytime residence, thereby contaminating the control treatments it does enable them to behave naturally and therefore appears to be a valid method of assessing the ability of synthetic pheromone blends or earwig exposed substrates to initiate aggregation behaviours in "real-life" scenarios.

Numerous HCs were identified from the cuticular washes of all life-stages and both adult sexes. Analysis of the cuticular HC profiles of adult male and female and 4th instar juveniles showed that the profiles of these differ from one another. These results also indicate the potential importance of HCs in other earwig behaviours besides those highlighted during this study and that of Mas & Kolliker (2011). The two alkatrienes 3,6,9-pentacosatriene and 3,6,9-heptacosatriene identified during this study were only isolated from adult males soon after the imaginal moult and so may have an important role in *F. auricularia* courtship. An assessment of these compounds may further aid understanding of earwig behaviours.

In conclusion, we provide first evidence that unsaturated cuticular HCs including (*Z*)-9-tricosene, (*Z*)-7-tricosene, (*Z*)-9-pentacosene and (*Z*)-7-pentacosene may mediate the formation of *F. auricularia* aggregations. However, whether the variable behavioural responses to these unsaturated HCs demonstrated in this study are due to pheromone plasticity or due to a minor component not being incorporated into the synthetic blend is currently unclear. If the aggregation pheromone production and response is indeed plastic in *F. auricularia* it may well explain the difficulties observed during previous attempts at its isolation. Similarly, it may also complicate the use of this pheromone when used as a method of controlling pestiferous earwig populations in agricultural and urban areas.

ACKNOWLEDGEMENTS

We wish to thank Andrew Smith for the use of his apple and cherry orchard, Ross Corkrey for his assistance in the recursive partitioning analysis and Dr Jason Smith for the synthesis of the unsaturated hydrocarbons used during this project. We also acknowledge Nicole Zhang

for her assistance in field data collection. This research was possible due to funding from Horticulture Australia Limited research grant MT 09006.

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Chapter 6 Can fluctuations in cuticular hydrocarbons explain the seasonal behaviour of a subsocial insect?

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Abstract – Cuticular hydrocarbons (HC) have been increasingly observed to provide a complex source of information that mediate numerous behaviours between individuals including species and kin recognition, sex determination, social dominance and reproductive status. The European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) is a cosmopolitan insect species found in many temperate regions worldwide. Earwigs exhibit a complex life-cycle, which includes maternal care, aggregation behaviours and the formation of mating pairs in subterranean nests prior to over-wintering. Seasonal earwig population monitoring has also demonstrated that earwig trap catches rapidly decline after their final juvenile moult. Recently, unsaturated cuticular HCs have been implicated in earwig aggregation behaviours. We investigate whether this decline in earwig trap catches is linked to fluctuations in earwig cuticular HC profiles and whether this decline relates to the differing behaviours in the field. This was achieved by sequentially sampling field collected earwigs over a 21 week field season and quantifying 51 cuticular HCs using gas-chromatography mass-spectrometry, while monitoring the seasonal decline in earwig trap catches. Our results show that earwig cuticular HCs do indeed fluctuate throughout their activity season. In female earwigs, the concentration of long-chain methyl-branched HCs greater than 27 carbon atoms in length increased > 1000-fold toward over-wintering. In males, these compounds were observed to diminish. We also demonstrate that production of the unsaturated cuticular HCs, (*Z*)-9-tricosene, (*Z*)-7-tricosene, (*Z*)-9-pentacosene, (*Z*)-7-pentacosene and (*Z*,*Z*)-6,9-pentacosadiene, which have previously been hypothesised to be *F. auricularia*'s aggregation pheromone components declined in both sexes from (mean ± SEM) 137.6 ng (± 30.9) in newly moulted males to 3.1 ng (± 0.8) in over-wintering individuals and from 37.3 ng (± 3.8) in newly moulted females to 1.4 ng (± 0.5) in over-wintering females. We also demonstrate that this decline in unsaturated HC production correlates strongly with the decline in earwig trap catches. We discuss whether the decline in earwig population estimates may be potentially linked to the timing of the formation of mating pairs and subsequent subterranean nesting behaviours, which may begin earlier in the season than previously reported.

Key Words - *Forficula auricularia*, Dermaptera, Aggregation pheromone, Plasticity, Aging, Alkenes, Methyl-branched hydrocarbons

INTRODUCTION

The European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae) is an invasive insect pest, native to Europe, western Asia and possibly Northern Africa (Lamb and Wellington 1975). Several accidental introductions in both the northern and southern hemispheres have led to its successful establishment in many temperate regions world-wide (Lea 1903; Crumb et al. 1941; Guillet et al. 2000b). Previously, climate and locality were believed to affect *F. auricularia* life-history (Wirth et al. 1998). High altitude populations were observed laying one clutch per season during early winter with a long gregarious adult phase and no diapause and those at lower altitudes laying two clutches per season with an imaginal overwintering diapause (Guillet et al. 2000a), the first clutch being laid at the beginning or end of winter with a second smaller clutch in late spring early summer (Lamb and Wellington 1975). However, genetic analysis of populations in Europe and North America identified two subspecies; subspecies A (laying one or two clutches per year) and subspecies B (laying two clutches per year) (Guillet et al. 2000a). Studies have also demonstrated that these populations co-exist in the wild with forced copulations between subspecies in the laboratory showing egg infertility that prohibits any genetic flow occurring between populations (Wirth *et al.* 1998).

In addition to these differences in reproductive strategy, *F. auricularia* displays various complex behaviours within its lifecycle, which differentiate it from other insect taxa including the formation of mixed aggregations containing both adult sexes and juveniles and maternal care (Lamb and Wellington 1975; Lamb 1976; Walker et al. 1993; Helsen et al. 1998). These behaviours have led to earwigs being increasingly considered a prime insect model to study the evolution of insect behaviours (Tomkins and Simmons 1998; Tomkins and Brown 2004; Mas and Kölliker 2011a; Mas and Kölliker 2011b).

In late autumn, male and female earwigs form pairs and excavate subterranean nests > 2 cm beneath the soil surface or under rocks and logs in preparation for overwintering (Lamb and Wellington 1975). Mating begins from late summer (Lamb and Wellington 1975) and continues through the overwintering phase (S. Quarrell, pers. obs.). Multiple mating has been observed in laboratory experiments but it remains unclear whether this occurs in field populations (Lamb 1976; Walker and Fell 2001). As mating may occur prior to nesting the male may not be the contributor of the paternal line (Brown 2006). Following nest formation

and mating the male exhibits mate guarding behaviours to prevent sneaky matings from other males and ensure paternity (Lamb 1976; Brown 2006). High male mortality is commonly observed during this over-wintering phase (Lamb 1976; Gingras and Tourneur 2001). Egg laying occurs mid to late winter, with any surviving males then aggressively evicted from the nest by the females soon after oviposition, after which time these males soon die (Lamb and Wellington 1975; Lamb 1976).

Female earwigs show strong maternal care for both eggs and young nymphs with eggs turned and cleaned to limit fungal infection (Kolliker and Vancassel 2007). Brooding females provide food throughout the first nymphal instar via two behavioural mechanisms either food regurgitation or by direct provisioning i.e. whole aphids (Staerkle and Kolliker 2008). First instar nymphs remain in the nest with the female until the end of the first moult, when both nymphs and females leave the nest to either nocturnally feed on vegetation and other insects then returning to the nest by day or leaving the nest permanently (Lamb and Wellington 1975). At this point in time the females of subspecies A will die and the females of subspecies B will establish another nest and lay again (Lamb and Wellington 1975). In orchards and forested areas free foraging earwigs are predominantly arboreal with earwigs residing under rocks, logs and within leaf litter where trees are not present (Lamb and Wellington 1975).

During this free foraging phase, earwigs form mixed aggregations that contain both adult sexes and all life stages, which are mediated via the use of an aggregation pheromone (Sauphanor 1992; Walker et al. 1993; Hehar 2007). However, these studies have failed to isolate the pheromone. One notable omission from these aggregation pheromone studies are the numerous cuticular hydrocarbons (HC) identified from female earwig cuticles by Liu (1991). Recently cuticular HCs were shown to be involved with maternal care behaviour, in particular, food provisioning to juveniles with juvenile HC composition fluctuating when food quality/ quantity was altered. These fluctuations were demonstrated to impact on the maternal care behaviour of nesting females (Mas et al. 2009; Mas and Kölliker 2011a) and the timing of future reproductive events (Mas and Kölliker 2011b). More recently, the cuticular HCs (*Z*)-9-tricosene, (*Z*)-7-tricosene, (*Z*)-9-pentacosene, (*Z*)-7-pentacosene have been implicated as the compounds, which mediate *F. auricularia* aggregations (see Chapter 5).

After the final juvenile moult, a rapid decline in adult earwig numbers in monitoring traps has been observed (Quarrell 2008; Moerkens et al. 2009). Moerkens et al. (2009) postulated that this decline reflects a real drop in the earwig population mediated by density dependent factors including reduced food availability, increased natural enemy populations, disease or the use of insecticides. However, this study was unable to confirm any of these hypotheses. An alternate cause for this decline, which was not hypothesised, is that high numbers of earwigs in traps is promoted by the active production of the aggregation pheromone and that the trap declines reflect the switching off of this pheromone interlinked with the formation of mating pairs earlier in the season than previously thought in the field. If this is the case this process may well be endocrine regulated as hormones have been shown to control insect reproductive cycles, species migration and pheromone production in insects (Barth 1965; Dukas and Mooers 2003; Schal et al. 2003). Indeed, juvenile hormone (JH) has already been shown to regulate the sexual maturity, reproductive cycles and maternal care instincts in the earwigs; *Euborellia annulipes* Lucas (Rankin et al. 1995a; Rankin et al. 1995b; Rankin et al. 1997), *Labidura riparia* Pallas (Baehr et al. 1982; Vancassel et al. 1984) and *Anisolabis maritima* Bonelli (Rankin et al. (1995a) cites Ozaki (1960)) and therefore may also regulate the production of *F. auricularia*'s aggregation pheromone. If the aggregation pheromone used by earwigs does decline throughout the season it may explain both the seasonal decline in earwig trap catches and the difficulty observed in isolating the earwig aggregation pheromone (Walker et al. 1993; Hehar 2007).

The aims of this study were to identify if any temporal fluctuations in the cuticular HCs of *F. auricularia* do occur throughout the earwig activity season, and to determine if these fluctuations correlate with the observed decline in earwig trap catches in the field and any changes in earwig behaviour. To do so we sequentially sampled the cuticular HCs of field-based earwigs while simultaneously monitoring earwig population dynamics.

METHODS AND MATERIALS

Chemical analysis

Six male and six female earwigs were collected from apple trees within a commercial apple orchard in the Huon Valley, Tasmania (Lat. 42˚ 59.755' S Long. 147˚ 4.328' E) every two weeks between the 16th December 2011 and the 20th March 2012. The earwigs were

collected by placing twenty corrugated cardboard rolls (8.5 cm x 9 cm) attached with garden twine (Zenith, REA 0060), at the base of each tree 30 cm above ground level. All earwigs were randomly selected from a variety of traps at each time point. Over-wintering individuals were collected from subterranean nests at the same site on the 9th May 2012.

Cuticular HCs were identified and quantified by immersing whole earwigs in 1 ml of hexane containing an *n*-C_{22} HC standard (50 µL; 25 µg in 1 ml) for one hour. Solvent extractions were then reduced under a gentle flow of nitrogen to ca. 100 µL and transferred into 150 µL Waters inserts (WAT 094171) for GC-MS analysis and stored at -6 °C until required. GC-MS analysis of hexane washes was performed with a Varian CP 3800 gas chromatograph, fitted with a Varian VF5-MS column (30 m, 0.25 mm, 0.25 um film thickness) coupled to a Bruker 300-MS triple quadrupole mass spectrometer in electron ionisation mode using 70 eV electrons. Samples were injected with a Varian CP-8400 autosampler into a Varian 1177 split/splitless injector at 270 °C with a 30:1 split ratio. Oven temperature was programmed from 50 ˚C (2 minute hold) to 150 ˚C at 30 ˚C per minute, then 150 ˚C to 300 ˚C at 8 ˚C/min (1 minute hold). Carrier gas flow was helium at 1.2 ml/minute using a constant flow mode. The MS was scanned from *m/z* 35 to 600 at 3 scans per second.

Methyl-branched hydrocarbons were identified as per chapter 5 using *n*-alkane standards, mass spectral fragmentation patterns from Doolittle *et al*. (1995) and Kroiss *et al*. (2011) and published retention index data from Carlson et al. (1998) and Katritzky *et al*. (2000). Double bond positions from alkenes and alkadienes were identified by derivatisation with dimethyl disulfide (DMDS) as per Carlson *et al.* (1989). Alkatriene double bond positions were determined using underivatised samples via mass spectral fragmentation patterns as described by Miller (2000) and Conner *et al*. (1980).

Earwig population monitoring

Earwig populations were monitored in a neighbouring apple block (ca. 50 m away) using corrugated cardboard rolls (8.5 cm x 9 cm) and at the same time points as stated above. The number, sex and life stage of each earwig found in the cardboard rolls was recorded and subsequently released at the tree base. The earwig traps were replaced fortnightly to prevent the aggregation pheromone from permeating into the trap and falsely inflating earwig population monitoring efforts (see chapter 5).

Statistical Analysis

Freidman's tests with Dunn-Bonferroni multiple pair-wise comparisons tests were performed on the earwig population data. To assess changes in individual cuticular HCs throughout the observation period Kruskal-Wallis tests with Dunn-Bonferroni multiple pair-wise comparisons tests were performed. Both Freidman's and Kruskal-Wallis tests were conducted using IBM SPSS Statistics version 19. To determine whether a correlation exists between the production of the HCs quantified and those behaviourally tested in Chapter 5 and the earwig trapping data Spearman's rho was also performed. To establish whether a relationship exists between the production of any single cuticular HC within the complete profile throughout the observation period and the number of earwigs found aggregating in trees, recursive partitioning was also performed by analysing the 51 HCs quantified together with the earwig trap catch data. Recursive portioning develops conditional inference trees. At each step a null hypothesis of no association is tested between the outcome and the covariates with the processing stopping if the null hypothesis is retained. If the null hypothesis is not retained the covariate with the strongest association is used to split the data into disjoint sets. This process is repeated until no covariate is associated with the data set. Recursive partitioning was performed using R version 2.15.1 using the "party" package and the "ctree" function.

RESULTS

Earwig population dynamics

The number of male, female and all juvenile earwigs observed in traps varied significantly over the field season (Figure 6-1A and 6-1B; Friedman's male $\chi^2 = 60.60$, $P < 0.001$; female $\chi^2 = 57.32$, $P < 0.001$; 4^{th} instars $\chi^2 = 133.15$, $P < 0.001$; 3^{rd} instars $\chi^2 = 69.80$, $P < 0.001$ and 2^{nd} instars $\chi^2 = 43.78$, $P < 0.001$). The earwig population displayed characteristics of subspecies B (Wirth et al. 1998) where two generations of 4^{th} instar juveniles are apparent peaking in numbers at or prior to the commencement of the observation period at week 1 with a second smaller generation peaking at ca. week 7 (Figure 6-1A). Male and females both peaked in number on the week 5 where a mean (± SEM) of 2.05 ± 0.46 males (range 0 – 9) and 2.35 ± 0.53 female earwigs (range 0 – 9) were observed per tree. A second smaller peak in adult numbers, though not statistically significant was also observed after the apple harvest in week 17 (Figure 6-1B; Bonferroni adjusted: males $Z = 3.003$, $P = 0.120$; females $Z = 2.977$, $P = 0.131$), which then significantly diminished by week 19 (Bonferroni adjusted: males $Z = 3.525$, $P = 0.019$; females $Z = 3.865$, $P = 0.005$).

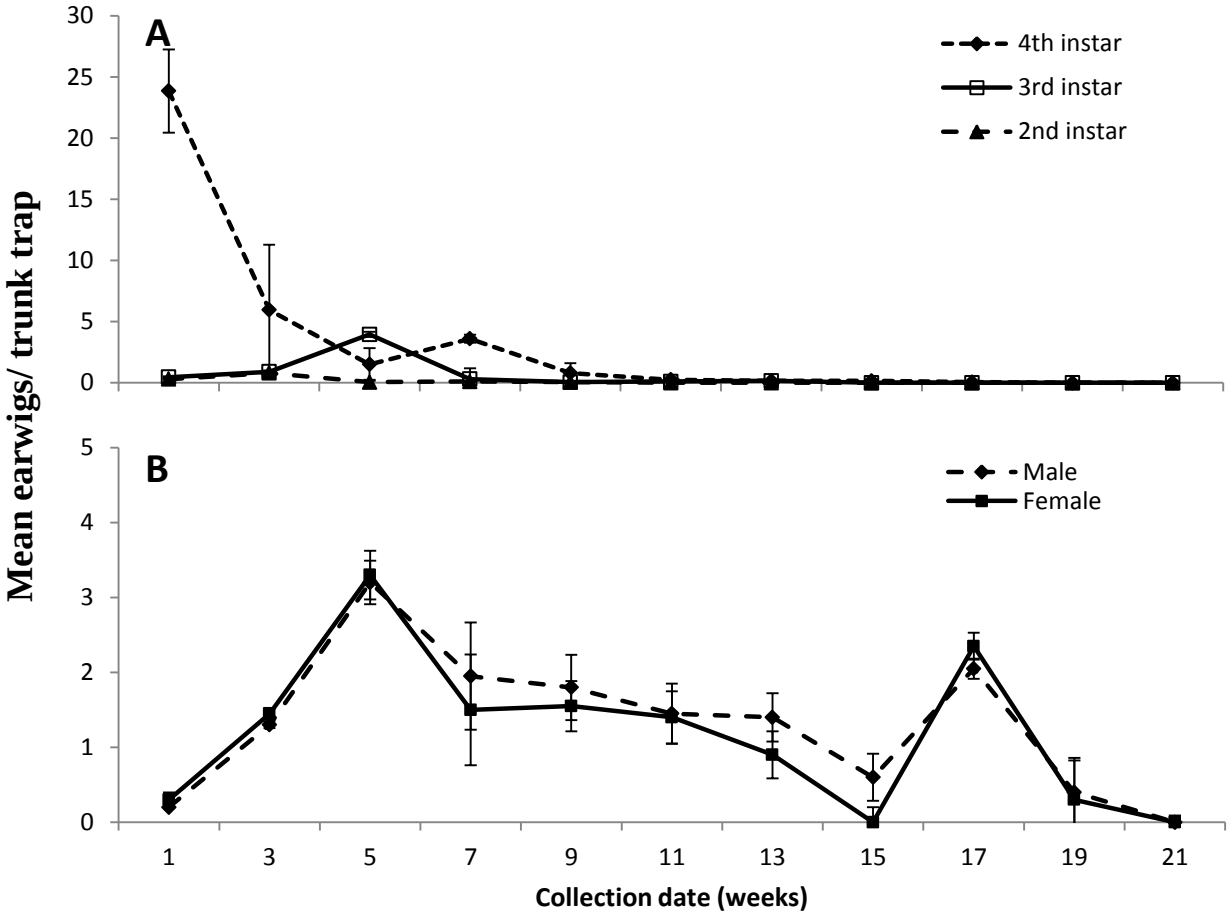

Figure 6-1. Mean (± SEM) *Forficula auricularia* per trap collected from apple trees (n = 20) from the 16th December 2011 to 5th May 2012 A) 2nd, 3rd and 4th instars earwigs per trap B) Adult male and female earwigs per trap.

HC analysis

A total of 51 cuticular HCs were identified from the hexane washed cuticles of male earwigs comprising alkanes, alkenes, alkadienes and alkatrienes varying from 21 to 31 carbon atoms in length and 49 HCs from the cuticles of female earwigs with neither of the alkatrienes, (*Z*,*Z*,*Z*)-3,6,9-C_{25} or (*Z*,*Z*,*Z*)-3,6,9-C_{27} recorded from the female cuticles (Table 6-1, Figures 6-2 and 6-3). The total HC concentration of adult male and female earwigs declined significantly between the start of the monitoring period in both males (Figures 6-2 and 6-3; Kruskal-Wallis $\chi^2 = 35.45$, df = 9, $P < 0.001$) and females (Kruskal-Wallis $\chi^2 = 35.05$, df = 9, $P < 0.001$). In males, the concentrations of only one HC, 3,7-diMe-C_{25} was observed not to have changed throughout the field season (Figure 6-3, Appendix 2; Kruskal Wallis; males χ^2

= 12.906, df = 9, $P = 0.167$). In females the production of this compound was observed to fluctuate significantly ($\chi^2 = 24.270$, df = 9, $P = 0.004$) where elevated levels were observed in early season (mean ± SEM; week 1 0.031 µg ± 0.008, week 3; 0.112 ± 0.077), late season (week 19; 0.156 ± 0.075) and in subterranean females (week 21; 0.125 ± 0.053). Despite the total HC profile concentration in females declining numerous long-chain methyl-branched HCs greater than 27 carbon atoms in length were observed to increase over 1000 times in concentration (Figure 6-3, Appendix 2)

Cuticular washes from recently moulted adult males collected in week 1 possessed significantly more HC (Figure 6-2; Mann-Whitney; $Z = -2.611$, $P = 0.008$) than recently moulted females (mean, *n*-C_{22} equivalents ± SEM; males 192.5 µg ± 3.4; females 77.2 µg ± 2.4). However, this trend was not consistently observed throughout the season with over-wintering males collected from within subterranean nests on week 21 having less HC (5.3 µg ± 0.7) than over-wintering females which contained 36.9 µg (± 9.8) of HC (Figure 6-2; Mann-Whitney; $Z = -2.562$, $P = 0.009$). Similarly, in week 15 males possessed significantly less HC than females (Mann-Whitney; $Z = -2.562$, $P = 0.009$) where males possessed (mean ± SEM) 7.971 µg ± 0.190 of HC compared to females which possessed 10.963 µg ± 0.251. Conversely, in week 17 a two-fold decline in HC was recorded in females resulting in males possessing more HC than females (Mann-Whitney; $Z = -2.402$, $P = 0.015$) with males possessing (mean ± SEM) 7.188 µg ± 0.193 of HC compared to females which possessed 5.033 µg ± 0.115. There was no significant differences in the quantity of cuticular HC between sexes in any of the other weeks (Mann-Whitney; week 3 $Z = -0.183$, $P = 0.931$; week 5 $Z = -1.826$, $P = 0.082$; week 7 $Z = -1.441$, $P = 0.180$; week 9 $Z = -1.121$, $P = 0.310$; week 11 $Z = -0.961$, $P = 0.394$; week 13 $Z = -0.183$, $P = 0.931$; week 19 $Z = -0.873$, $P = 0.937$).

The large quantities of two alkatrienes, (*Z*,*Z*,*Z*)-3,6,9-C_{25} (peak 13) and (*Z*,*Z*,*Z*)-3,6,9-C_{27} (peak 26) produced by males, but not females, was observed in its highest concentrations in week 1 (Figure 6-2; 0.17 µg ± 0.06 and 0.54 µg ± 0.07 respectively). However, the production of these compounds declined significantly throughout the season (Kruskal-Wallis: (*Z*,*Z*,*Z*)-3,6,9-C_{25} $\chi^2 = 30.492$, df = 9, $P < 0.001$; (*Z*,*Z*,*Z*)-3,6,9-C_{27} $\chi^2 = 37.596$, df = 9, $P < 0.001$). The majority of the decline in these compounds were observed in the four weeks after the final moult has occurred (Appendix 2; Bonferroni adjusted, (*Z*,*Z*,*Z*)-3,6,9-C_{25} $Z = 4.129$, $P = 0.002$; (*Z*,*Z*,*Z*)-3,6,9-C_{27} $Z = 4.684$, $P < 0.001$) with subterranean males at the end of the

season possessing 0.003 µg (± 0.001) and 0.006 µg (± 0.002) for both (*Z*,*Z*,*Z*)-3,6,9-C_{25} and (*Z*,*Z*,*Z*)-3,6,9-C_{27} respectively. The unsaturated HCs (*Z*)-7-C_{25} and (*Z*)-7-C_{27} were both observed to increase, though not significantly within the first 2 weeks after the final moult has occurred (Figure 6-4, Bonferroni adjusted; males (*Z*)-7-C_{25} Z = -1.850, P = 1.00; (*Z*)-7-C_{27} Z = -0.995, P = 1.00 ; females (*Z*)-7-C_{25} Z = 0.304, P = 1.00; (*Z*)-7-C_{27} Z = -0.458, P = 1.00). For all HC phenology data see Appendix 2.

Table 6-1. Complete list of compounds detected from the cuticles of *F. auricularia*.

Peak	Compound name	Abbreviation	Peak	Compound name	Abbreviatio
1	*n*-heneicosane	*n*-C_{21}	27	(*Z*)-7-heptacosene	(*Z*)-7-C_{27}
2	(*Z*)-9-tricosene	(*Z*)-9-C_{23}	28	*n*-heptacosane	*n*-C_{27}
3	(*Z*)-7-tricosene	(*Z*)-7-C_{23}	29	15-methylheptacosane	15Me-C_{27}
4	*n*-tricosane	*n*-C_{23}	29	13-methylheptacosane	13Me-C_{27}
5	7-methyltricosane	7Me-C_{23}	30	11-methylheptacosane	11Me-C_{27}
6	5-methyltricosane	5Me-C_{23}	31	9-methylheptacosane	9Me-C_{27}
7	3-methyltricosane	3Me-C_{23}	32	7-methylheptacosane	7Me-C_{27}
8	*n*-tetracosane	n-C_{24}	33	5-methylheptacosane	5Me-C_{27}
9	3,7-dimethyltricosane	3,7-diMe-C_{23}	34	11,15-dimethylheptacosane	11,15-
10	6,9-pentacosadiene	6,9-C_{25}	35	9,13-dimethylheptacosane	9,13-diMeC_{27}
11	(Z,Z)-6,9-pentacosadiene	(*Z*,*Z*)-6,9-C_{25}	36	3-methylheptacosane	3Me-C_{27}
12	(*Z*)-9-pentacosene	(*Z*)-9-C_{25}	37	*n*-nonacosane	*n*-C_{29}
13	(*Z*,Z,*Z*)-3,6,9-pentacosatriene	(*Z*,*Z*,*Z*)-3,6,9-C_{25}	38	15-methylnonacosane	15Me-C_{29}
14	(*Z*)-7-pentacosene	(*Z*)-7-C_{25}	39	13-methylnonacosane	13Me-C_{29}
15	*n*-pentacosane	*n*-C_{25}	40	11-methylnonacosane	11Me-C_{29}
16	13-methylpentacosane	13Me-C_{25}	41	9-methylnonacosane	9Me-C_{29}
17	11-methylpentacosane	11Me-C_{25}	42	7-methylnonacosane	7Me-C_{29}
18	9-methylpentacosane	9Me-C_{25}	43	11,15-dimethylnonacosane	11,15-
19	7-methylpentacosane	7Me-C_{25}	44	9,13-dimethylnonacosane	9,13-diMeC_{29}
20	5-methylpentacosane	5Me-C_{25}	45	3-methylnonacosane	3Me-C_{29}
21	3-methylpentacosane	3Me-C_{25}	46	15-methylhentriacontane	15Me-C_{31}
22	*n*-hexacosane	*n*- C_{26}	47	13-methylhentriacontane	13Me-C_{31}
23	3,7-dimethylpentacosane	3,7-diMe-C_{25}	48	11-methylhentriacontane	11Me-C_{31}
24	6,9-heptacosadiene	6,9-C_{27}	49	9-methylhentriacontane	9Me-C_{31}
25	(*Z*)-9-heptacosene	(*Z*)-9-C_{27}	50	11,15-dimethylhentriacontane	11,15-
26	(*Z*,Z,*Z*)-3,6,9-heptacosatriene	(*Z*,*Z*,*Z*)-3,6,9-C_{27}	51	9,13-dimethylhentriacontane	9,13-diMeC_{31}

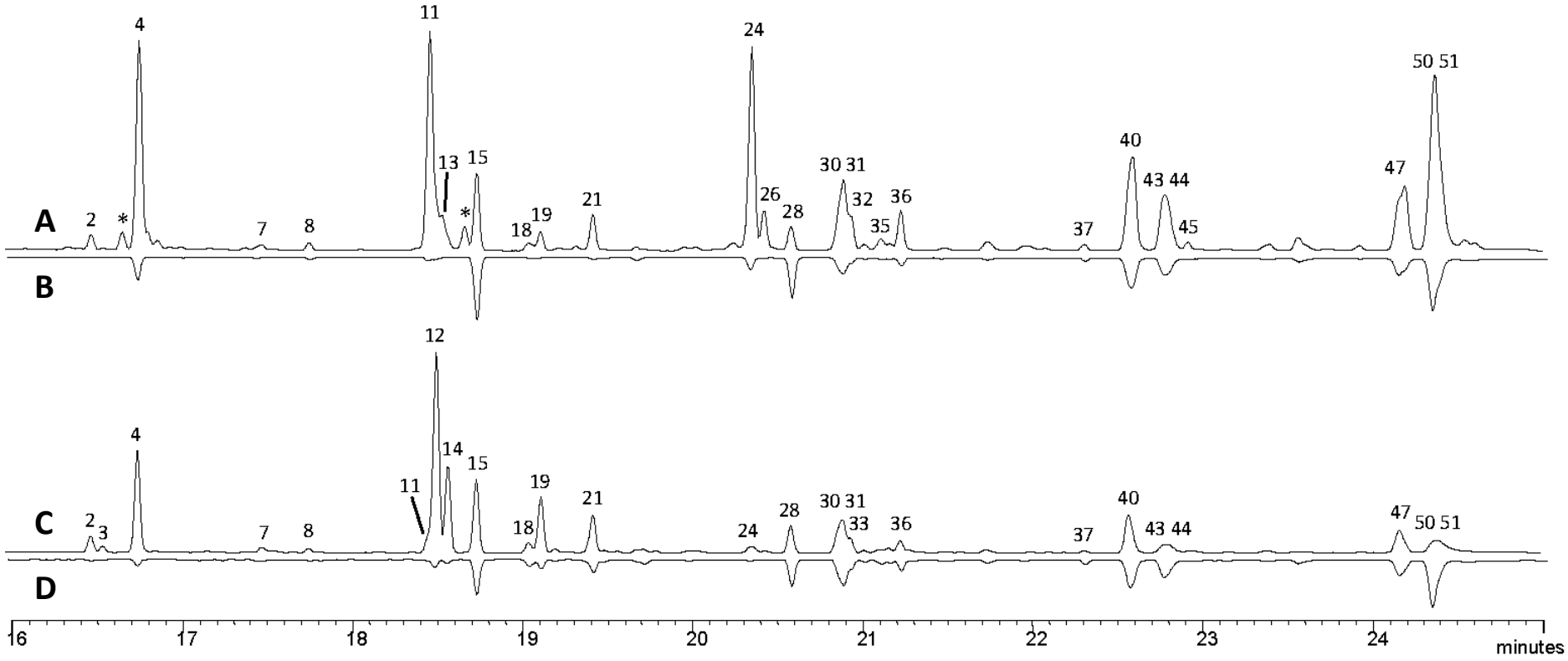

Figure 6-2. Representative gas-chromatograms of *Forficula auricularia* cuticular hydrocarbons collected from **A)** a recently moulted male **B)** an over-wintering male collected from a subterranean nest **C)** a recently moulted female **D)** an over-wintering female collected from a subterranean nest. Numbers above peaks refer to compounds listed in Table 6-1.

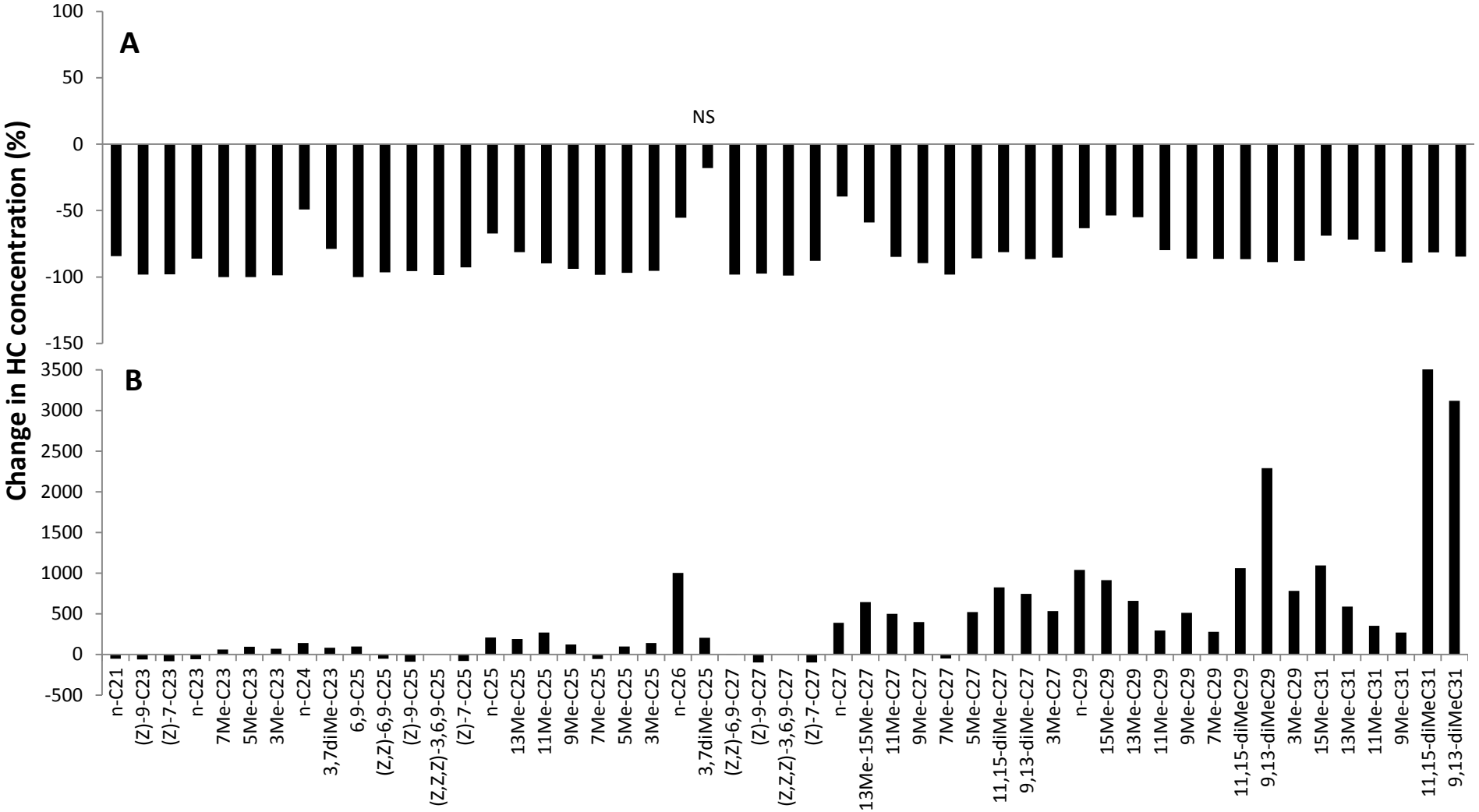

Figure 6-3. Mean percentage change in *Forficula auricularia* cuticular HC composition between recently moulted and over-wintering adult **A)** males and **B)** females. Six male and six female earwigs were collected and analysed at each time point. Negative values indicate a decline in HC production. Positive values indicate an increase in production. All HCs were observed to change over time unless otherwise indicated (Kruskal-Wallis; $P < 0.05$). NS indicates no significant difference. For all HC quantities (µg) and *P*-values see Appendix 2.

Recursive partitioning did not identify a relationship between a specific compound(s) and the decline in earwig catches within traps when analysed by sex or by month (Appendix 3, $P > 0.05$). However, differences were detected when sexes were pooled together with the conditional inference regression tree indicating that a relationship exists between several cuticular HCs and the total number of earwigs caught in traps throughout the earwig activity season (Figure 6-4A). All of the HCs specified in the inference tree declined throughout the earwig activity season in both sexes (Kruskal-Wallis: 7Me-C_{27} males $\chi^2 = 25.57$, df = 9, $P = 0.002$; females $\chi^2 = 36.55$, df = 9, $P < 0.001$; (*Z*)-7-C_{27} males $\chi^2 = 37.34$, df = 9, $P < 0.001$; females $\chi^2 = 43.37$, df = 9, $P < 0.001$; (*Z*)-7-C_{25} males $\chi^2 = 41.31$, df = 9, $P < 0.001$; females $\chi^2 = 31.56$, df = 9, $P < 0.001$; *n*-C_{23} males $\chi^2 = 31.68$, df = 9, $P < 0.001$; females $\chi^2 = 32.01$, df = 9, $P < 0.001$ and 3Me-C_{29} males $\chi^2 = 39.41$, df = 9, $P < 0.001$; females $\chi^2 = 39.40$, df = 9, $P < 0.001$).

The conditional inference regression tree (Figure 6-4A), first divides a population (Node 1) of 9 individuals from the remaining earwigs analysed based on having 7Me-C_{27} at concentrations > 0.217 µg. Figure 6-4B shows these this concentration of 7Me-C_{27} only occurred in the weeks prior to week 4 (Bonferroni adjusted, $Z = 3.452$, $P = 0.025$) when the greatest number of earwigs were found within traps (Figure 6-1). Node 2 indicates the population is further divided by individuals (n = 7) possessing (*Z*)-7-C_{25} at concentrations > 0.966 µg all of which occurred during peak aggregation period (Figures 6-1 and 6-4). Node 3 identifies 57 individuals that contain (*Z*)-7-C_{27} at concentrations < 0.004 µg, which again occurred after the week 6 (Figure 6-4B; Bonferroni adjusted, $Z = 4.279$, $P < 0.001$). The remaining individuals are split within nodes 5 and 6. Node 5 splits 7 individuals based on concentrations of *n*-C_{23} > 0.417 µg, which again declined significantly throughout the season, with the majority of the decline occurring between week 1 and week 6 (Figure 6-4B; Bonferroni adjusted, $Z = 4.039$, $P = 0.002$). The remaining individuals (n = 36) are those possessing < 0.417 µg of *n*-C_{23} and are subsequently divided by node 6 based on possessing concentrations of 3Me-C_{29} more or less than 0.016 µg, which occurred at both the beginning and end of the activity season (Figure 6-4B). With the exception of some outliers, the box plots in Figure 6-4A show little variation within each terminal node when the variation is expressed as a proportion of the total variation of the HCs flagged by the conditional inference regression tree.

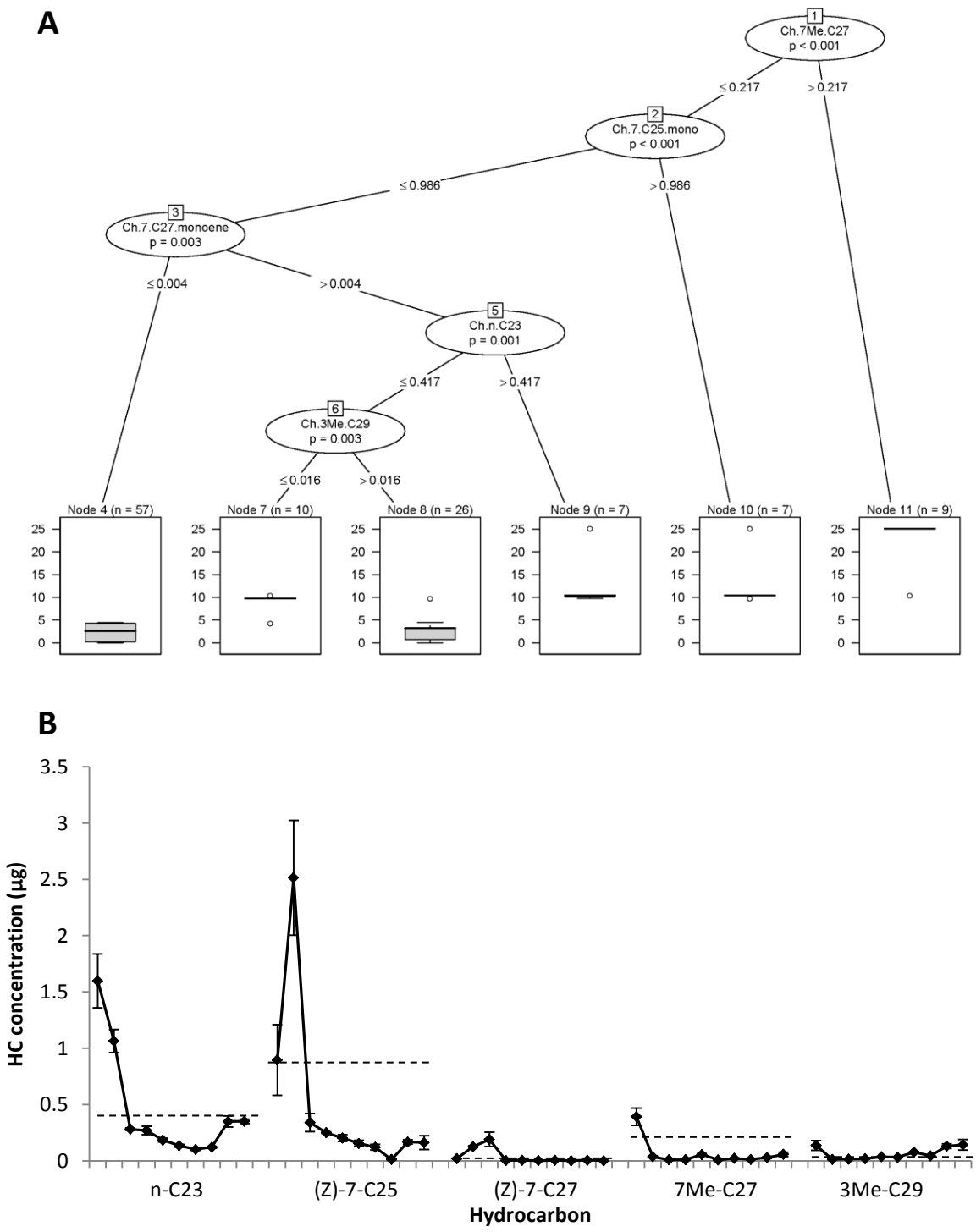

Figure 6-4. A) Recursive partitioning conditional inference decision tree highlighting the relationship between the concentrations of adult *Forficula auricularia*'s cuticular HCs when pooled together by sex and the total number of earwigs caught in earwig traps at the same time points. The number of individuals within each terminal node is denoted by the *n*-value above each box plot. The box plots signify the level of variation of each HC within each terminal node and are expressed as a proportion of the total variation within the HCs identified as important within the conditional inference regression tree. **B)** Mean (SEM) temporal fluctuations of the cuticular HCs identified by the conditional inference decision tree. Dotted lines indicate the threshold for each compound indicated in the decision tree. Fortnightly sampling dates are expressed from left to right for each compound.

The production of the unsaturated HCs assessed for behavioural activity in Chapter 5 were all demonstrated to decline significantly from early adulthood to over-wintering (Figure 6-5) (*Z*)-9-C_{23} (Kruskal-Wallis; male $\chi^2 = 36.638$, df = 9, $P < 0.001$; female $\chi^2 = 27.140$, df = 9, $P < 0.001$), (*Z*)-7-C_{23} (Kruskal-Wallis; male $\chi^2 = 36.052$, df = 9, $P < 0.001$; female $\chi^2 = 27.140$, df = 9, $P < 0.001$), (*Z*,*Z*)-6,9-C_{25} (Kruskal-Wallis; male $\chi^2 = 32.558$, df = 9, $P < 0.001$; female $\chi^2 = 24.409$, df = 9, $P = 0.004$) and (*Z*)-9-C_{25} (Kruskal-Wallis; male $\chi^2 = 42.613$, df = 9, $P < 0.001$; female $\chi^2 = 26.281$, df = 9, $P = 0.002$). When these unsaturated HCs are pooled together their production declines from (mean ± SEM) 1.9 µg (± 0.2) to 0.1 µg (± 0.02) in males and from 4.4 µg (± 1.5) to 0.6 µg (± 0.04) in females. However, in males the production in these unsaturated HCs was observed to increase to 4.3 µg (± 1.24) and then significantly decline in week 5 to 0.7 µg (± 0.2) though not significantly, suggesting a second adult generation (Bonferroni adjusted, $Z = 0.057$, $P = 1.000$ and $Z = 1.829$, $P = 1.000$) respectively.

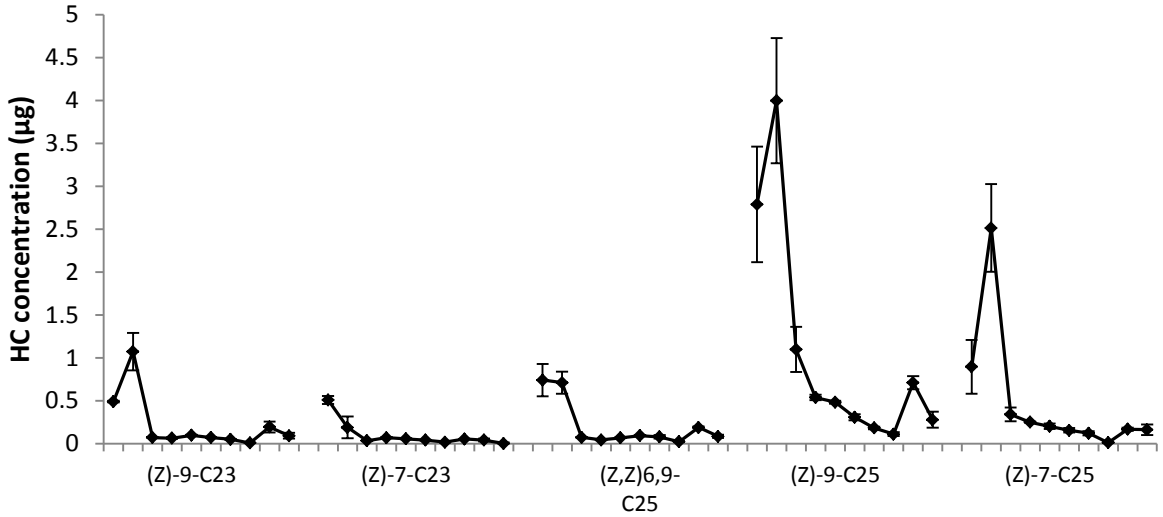

Figure 6-5. Mean (± SEM) temporal fluctuation of cuticular HCs hypothesised to be *Forficula auricularia* aggregation pheromone components when pooled by sex (see chapter 5). Fortnightly sampling dates are expressed from left to right for each compound.

When the unsaturated HC production from both sexes is pooled together as would be observed in mixed sex aggregations in the field, their production declines significantly (Kruskal-Wallis, $\chi^2 = 54.25$, df = 9, $P = 0.015$) from (mean ± SEM) 4.37 µg (± 1.57) in recently moulted individuals to 0.56 µg (± 0.32) in those found within subterranean nests. This decline in unsaturated HC production correlates significantly with the number of

earwigs found within trunk traps (Figure 6-6; Spearman's rho = 0.736, n =11, $P = 0.010$). No significant differences were observed between the unsaturated HCs fraction from those individuals collected within the first 9 weeks and those collected in week 19 (Figure 6-6; Bonferroni adjusted, $P > 0.05$). Similarly, no difference was observed in the unsaturated HCs collected from earwigs in week 17 and the earwigs collected from subterranean nests in week 21 (Figure 6-6; Bonferroni adjusted, $Z = -1.730$, $P = 1.00$).

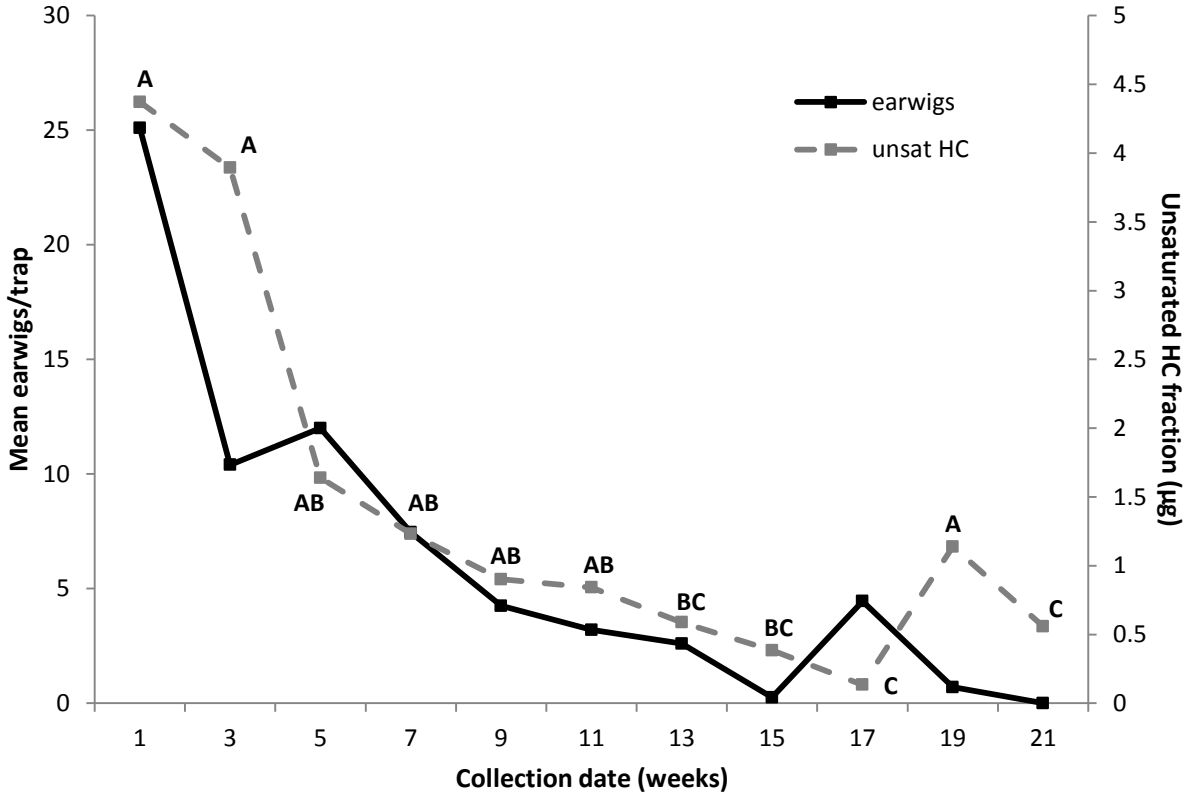

Figure 6-6. Mean number of earwigs found in earwig traps and unsaturated HC fraction when pooled by sex of the total HC profile of male and female *Forficula auricularia* demonstrated to have behavioural activity in Chapter 5. Letters indicate significant differences in temporal production of unsaturated HCs (Bonferroni adjusted $P < 0.05$).

DISCUSSION

The analysis of cuticular HCs identified from *F. auricularia* throughout the summer/ autumn period shows that temporal fluctuations do occur and that the decline in unsaturated HC production in particular the alkenes previously demonstrated to be attractive to earwigs correlate strongly with the decline in aggregation sizes within traps in the field. However,

whether these are the result of aging or due to endocrine regulation of cuticular HC production remains unclear.

Insect aging is being increasingly recognised as impacting cuticular HC composition in insects (Hugo et al. 2006; Nunes et al. 2009; Kuo et al. 2012). Indeed, Hugo et al. (2006) used the decline in mosquito cuticular HCs to determine the age of *Aedes aegyptii* in wild populations. Our results showed that in females, the shorter-chain HCs decreased overtime whilst the longer-chain HCs increased significantly in concentration. This trend was also observed in the stingless bee (*Frieseomelitta varia*) (Nunes et al. 2009) although the reasons for this are not entirely clear. However, in earwigs, one possibility is that physiological requirements of the integument during overwintering in subterranean nests differ to those within aggregations due to over-wintering earwigs having to endure excessive cold and moisture (Gingras and Tourneur 2001). Indeed, the increase in longer-chain HCs observed in females, but not males, may play a role in the higher rate of over-wintering survival in females compared to males in other earwig studies (Lamb 1976; Gingras and Tourneur 2001). If this is the case it would be expected that the earwigs may down-regulate the production of the aggregation pheromone components after mating or mate selection has occurred, as they are energetically costly to produce (Rantala et al. 2003) and redirect their energy to the production of HCs that would aid survival within the soil over winter.

If this is the case it may explain the observations of Moerkens et al. (2009) who postulated density dependent factors including migration, pathogens, predation, parasitoids and parasites are the cause of the decline in trapped earwig populations. In *F. auricularia*, mating is commonly regarded as occurring from late summer to early autumn (Lamb and Wellington 1975) and continues through the overwintering phase (S. Quarrell, pers. obs.) with nest formation beginning in late autumn (Lamb 1976). However, our results indicate that the most likely cause for this decline is the formation of mating pairs, from early adulthood onward and their subsequent movement into subterranean nests. If this is the case it would be expected that the population decline would be greater in single brood populations than in double brood populations, which though not explicitly stated does appear to have occurred in the Moerkens et al. (2009) study. Although mating status has been observed to impact on the production of cuticular HCs in other insects such as *Drosophila melanogaster* (Everaerts *et al.* 2010) it has yet to be investigated in earwigs.

The two alkatrienes, (*Z*,*Z*,*Z*)-3,6,9-C_{25} and (*Z*,*Z*,*Z*)-3,6,9-C_{27} identified in males alone were only isolated in high concentration soon after the final moult. These results coupled with the decline in trap catches increase the possibility that they may play an important role in *F. auricularia* courtship. However, as to why these compounds appear to diminish so rapidly in new adults remains unclear, unless the low concentrations observed in the weeks following this period are sufficient to illicit a response from females.

There was a strong correlation between the unsaturated cuticular HCs implicated as *F. auricularia*'s aggregation pheromone components and earwig trap catches, again suggesting that unsaturated HC production may indeed be involved in formation of earwig aggregations. However, environmental stimuli including conspecific interactions have been implicated in pheromone production and/or emission in other insects (Moore et al. 1995; Thomas et al. 2011; Thomas and Simmons 2011). If this is case, an alternative explanation for our findings is that HC production is regulated by population density (interaction with conspecifics) where unsaturated HCs are down-regulated when in low population densities. Although neither JH nor conspecific interactions were empirically tested, a 2 week delay in decreasing unsaturated HC production and trap catch numbers is clearly evident. Indeed, the observed increase in aggregation size post apple harvest and unsaturated HC fraction in the two weeks following would indicate that age or reproductive status are a possible causes for the decline in both variables.

These processes are most likely endocrine regulated. The importance of hormones such as JH has on the regulation of reproductive cycles, species migration and pheromone production has been long recognised in many other insect species (Barth 1965; Dukas and Mooers 2003; Schal et al. 2003; Jurenka 2004). In earwigs, JH has already been implicated in maternal care behaviours and reproductive cycles (Vancassel et al. 1984; Rankin et al. 1997) but, again, the role it plays in aggregation pheromone production remains to be proven. However, it would appear that the production of the two alkatrienes by males, coupled with the rapid dispersal of adults thereafter (Moerkens et al. 2009) as sexual maturity increases and mating begins (Lamb and Wellington 1975) would lead to the assumption, that the reduction in trap catches (aggregation sizes) are linked to the reproductive status of earwigs. It is therefore also likely that formation of aggregations by adults and juveniles may be driven by differing factors to that of juveniles, which may aggregate initially to take full advantage of the female's maternal behaviours and later to minimise the possibility of predation (Hamilton 1971).

Whereas, the formation of adult aggregations in early adulthood may be to enable the formation of mating pairs prior to over-wintering, which would explain earwig dispersal (reduction in trap catches) soon after the imaginal moult.

Adult numbers were also observed to increase after the apple harvest during week 17 presumably due to the removal of apple bunches, which are known to be daytime residences of *F. auricularia* (Nicholas et al. 2005). This increase in population coincided with an increase in cuticular HCs including a small increase in the production of the alkatriene 3,6,9-C_{27} in males and methyl-branched HC, 3,7-diMe-C_{25} in females, which were both previously shown in Chapter 5 to be distinguishing features between male and female cuticular HC profiles. Although it is unclear whether these individuals were derived from the either the first or second generations it is apparent that the arboreal population was producing levels equivalent to that found early in the season. This lends weight to the possibility that they are indeed from the later generation and subsequently lag behind the earlier generation in the formation of mating pairs and subsequent nesting behaviours.

This study highlights how complex the behaviour and chemical communication system of *F. auricularia* is and that cuticular HCs appear likely to play a significant role in mediating numerous earwig behaviours including maternal care, mate finding and aggregation. Our results also emphasize that a great deal of further research is needed if a better understanding is to be attained with respect to earwig aggregation behaviours and whether a relationship exists between aggregation, cuticular hydrocarbons, JH production and any subsequent changes in reproductive status.

ACKNOWLEDGEMENTS

We wish to thank Andrew Smith for the use of his orchard and Dr Ross Corkrey for his assistance in the recursive partitioning analysis. This research was funded by Horticulture Australia Limited (research grant MT 09006) with the industry levies from Cherries Australia Inc. and Apple and Pear Australia Ltd. and matched funds from the Australian Government.

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Chapter 7 General Discussion

This thesis presents several findings that may have considerable impact on how *F. auricularia* is viewed in both apple and cherry orchards and provides first evidence that the aggregation pheromone used by *F. auricularia* is comprised of unsaturated cuticular HCs. It also demonstrates that Australian and New Zealand earwig populations consist entirely of subspecies B, which produce two generations per year. These populations appear to have been established by few individuals and have then gone on to successfully spread through the temperate regions of both countries, despite their limited genetic diversity. Although not found during the limited collections made in this study further surveys of native ecosystems to confirm the absence of European earwigs from these ecosystems is prudent. The implications for Australian cherry orchards if they were ever to contain the differing European earwig subspecies would appear to be minimal as it is the larger, first earwig generation that is injurious to the fruit, with the second, smaller generation emerging after the cherry harvest. In general however, subspecies B populations with their second generation may be expected to be active in the field longer than subspecies A populations. This may create issues in crops that are harvested in late summer through to autumn such as agronomic crops, stonefruits, raspberries and wine grapes where earwig subspecies B population dynamics will add to their increasingly recognised pest status (Bower 1992; Kehrli *et al.* 2012; Mangano & Severtson 2012).

The spread of *F. auricularia* following invasion and subsequent distribution throughout the south-eastern states of Australia, signal that it is highly likely that *F. auricularia* will continue to invade the broad-acre production areas of Western Australia. In south-west Western Australia, *F. auricularia* are already considered a pest in canola, wheat and barley where they are commonly found aggregated in crop residues, feed on the emerging cotyledons of establishing crops and later, creating contamination issues at harvest. This has led to the increased use of broad-spectrum insecticides with limited control commonly observed (Mangano & Severtson 2012). It is therefore vital that the potential distribution of *F. auricularia* in Western Australia be determined. Furthermore, action thresholds should be developed for its control in broad-acre cropping systems to minimise the impact that this insect has on crop establishment and product contamination at harvest. There also exists a window of opportunity to survey the levels of other invertebrate fauna in differing agro-ecosystems in Western Australia pre and post earwig invasion to better understand the impact of earwig introduction into these systems.

The aggregation behaviour of *F. auricularia* appears to exacerbate earwig damage levels in sweet cherry crops where they can be found in considerable numbers within large, tightly packed cherry bunches. However, the ensuing damage appears to differ greatly between cherry cultivars for example Sweet Georgia damage was approximately five times more likely to occur than that observed in Lapin, despite Sweet Georgia being a sport developed from a Lapin mutation. Similarly, damage type can also differ between cultivars with stem damage most prevalent in Ron's Seedling, with 30-60% stem damage irrespective of bunch size. Why these differences occur between cultivars may include sugar, organic acid or phenolic compositions, which do differ between cherry cultivars (Gonçalves *et al.* 2004; Kelebek & Selli 2011; Liu *et al.* 2011). Indeed, phenolic concentration has been shown to impact on the feeding preference of cranberry cultivars by a number of insect pests including several Coleoptera and Lepidoptera (Neto *et al.* 2010). Although developing a greater understanding of cherry preferences may enable producers to select against cherry cultivars that earwigs prefer, it may be that selection for traits to deter earwigs may increase the impact of a currently minor secondary pest species which prefers the 'earwig resistant' cultivar as has been observed in other pest insects (Ayres *et al.* 1997).

The results of our intraguild compatibility study concur with the results of Nicholas *et al.* (2005) who showed that *F. auricularia* are beneficial WAA predators. Hence, efforts should be made to maintain high earwig numbers wherever practical. However, if WAA control is to be achieved without targeted insecticide applications we also demonstrate that sites under very high WAA pressure also require *A. mali* to achieve control. Limited indirect evidence of intraguild predation of *A. mali* by earwigs was also evident early in the season in trees with high early season *A. mali* numbers and high third instar earwig numbers. A more targeted study, during this early season time window, investigating the level of *A. mali* predation by earwigs utilising selective exclusion of each species and/or the genetic analysis of earwig gut contents may further elaborate on the frequency of earwig predation of *A. mali* and its impact on WAA infestation levels observed at harvest.

Our earwig estimates in apple orchards relate to subspecies B earwig populations, which produce two clutches of eggs per season, whereas subspecies A populations lay either one or two clutches per season. However, because first generation earwig and *A. mali* numbers were shown to be the key to attaining effective WAA control, our estimates may well stand irrespective of the earwig subspecies. This is especially so as the second *F. auricularia*

generation was observed to be far smaller than the first generation, as was also reported by Moerkens *et al.* (2009). The WAA and *A. mali* populations monitored here were within a cool temperate region of Australia with fewer generations of both WAA and *A. mali* observed compared to other studies in warmer climates (Asante & Danthanarayana 1992; Goossens *et al.* 2011). Therefore, it is recommended that field validation of these beneficial insect thresholds should be conducted in a variety of climates to ensure they are robust in all apple growing regions.

Earwig trap catches were observed to decline steadily in the latter half of the season. This observed decline in trap catches correlates with a decline in cuticular hydrocarbons (HC) produced by adults soon after the imaginal moult. Analysis of substrates exposed to earwigs and the solvent washes of individuals also suggest cuticular HCs appear to mediate earwig aggregations as they were the only earwig derived compounds found. Both substrates and a suite of unsaturated HCs were subsequently shown to be attractive to both adult sexes and all free-foraging juvenile life stages. Indeed, this is the first report of the successful isolation and response to a synthetic pheromone in any Dermaptera. Although the synthetic alkene blends failed to always attract significantly higher numbers of earwigs in all field tests compared to solvent controls, two-fold increases in earwig trap catches were observed on numerous occasions. A similar two-fold increase in trap catches was also observed to the pre-exposed earwig traps when compared to their control treatments (Chapter 5, Table 5-5). Unfortunately, these experimental treatments were not tested on the same date with the same blends and it therefore confounds understanding how equivalent our synthetic blends are to a substrate pre-exposed to *F. auricularia*. Why there was a level of inconsistency in earwig response to the synthetic blend remains unresolved with either the synthetic pheromone blend missing a component or that pheromone production and earwig response varies with earwig age and phenology. Indeed, either of these hypotheses remain plausible, as the alkadienes (*Z*,*Z*)-6,9-C_{25} and (*Z*,*Z*)-6,9-C_{27} were not tested due to sourcing problems and the unsaturated cuticular HC fractions from both adult sexes did vary over time. Our four component blend, which contained (*Z*)-9-tricosene, (*Z*)-7-tricosene, (*Z*)-9-pentacosene, (*Z*)-7-pentacosene had variable attraction to adults but its ability to induce juvenile aggregations appeared more consistent. Certainly, further testing and blend development is required before the pheromone could be made commercially available.

Why *F. auricularia* aggregate is currently unknown and may differ between adults and juveniles. Walker *et al*. (1993) hypothesised that a number of functions may be served by earwig aggregations including mate finding in adults, predator defence and increased juvenile growth and development. The results from this study concur with this hypothesis where attraction to substrates was dependent on which sex or life stage the substrate was pre-exposed to and the sex or life-stage of the test subject. Indeed, if juveniles are aggregating to enhance their survival and growth they would be attracted to all members of the population as was observed in this study. Similarly, if adults utilise aggregation during early adulthood to find mates they may be expected to disperse after mates have been located, which also appears to have occurred here. Finally, relatively few adults were observed within the trunk traps or cherry bunches, with the largest aggregations dominated by juveniles, which again adds support to this hypothesis.

What mate finding behaviours adult *F. auricularia* exhibit soon after reaching adulthood is unknown, however, based on trap catches adult dispersal is apparent, with adult numbers relatively small compared to that of juveniles. The potential for rapid adult dispersal is further supported by the lack of a peak in second generation adult numbers in both the intraguild compatibility study and the HC sequential sampling trials. However, the presence of the second generation adults, which may still exhibit aggregation behaviour, could explain the small increase in both earwig numbers and corresponding increase in cuticular HCs observed during the sequential sampling of earwig trap catches and cuticular HCs (Chapter 6, Figure 6-6). Similarly, due to the potential overlap of older first and younger second generations of adults in the field at the same time, differences in the population response to the synthetic pheromone may result. The simpler population to study pheromone responses would be populations of the one generation subspecies A earwigs where no such overlap would occur. Alternatively, developing a method to age earwigs collected in the field within pheromone traps using features such as ovarian status or cuticular growth (Hayes & Wall 1999) may also prove fruitful. Similarly, the manipulation of juvenile hormone titres, which has already been shown to control maternal care instincts and oogenesis in female earwigs (Rankin et al. 1997) may help to resolve the relationship between earwig age and pheromone production and response. If aggregation pheromone production and subsequent aggregation behaviours are endocrine regulated, the observed down regulation in unsaturated HC production should be inversely correlated with sexual maturity in adults. The response of adults to aggregation pheromone could also be investigated by assessing the response of adults of known age to

both earwig exposed substrates and/or the synthetic pheromone as older adults may not be expected to respond to the aggregation pheromone as strongly as sexually immature adults may.

Whether the cuticular HC profiles between the two subspecies of *F. auricularia* differ and if the same aggregation pheromone is used by the two subspecies also needs to be investigated. There have been a handful of studies on other insects that suggest HC or pheromone differences are possible. The cockroach *Cryptocercus punctulatus* Scudder found within the Appalachian Mountains is divided into a complex of four distinct sibling species based on differing chromosome numbers with two of these four sibling species possessing the same HC profiles (Everaerts *et al.* 2008). Similarly, differences have also been observed in the sex pheromone blends of *Drosophila melanogaster* Meigen mutants with only a minor mutation sufficient to illicit a shift in pheromone production (Marcillac *et al.* 2005).

The findings of this thesis have significant relevance to many agricultural industries aside from just apple and sweet cherry production. The potential benefits of maintaining *F. auricularia* populations in crops where earwigs are beneficial to minimise pest outbreaks is evident from the apple study. By doing so farmers may not only increase farm profits by reducing crop losses and increasing plant health, but also by reducing insecticide usage. Similarly, it also shows that high earwig numbers in cherry orchards may not always cause significant crops losses as the level of damage observed is highly cultivar dependent. Furthermore, first evidence is presented that cuticular HCs play a key role in the aggregation behaviour of *F. auricularia*, which once fully understood could lead to earwigs becoming a useful insect model to examine maternal care and endocrine regulation of pheromone signals in insects.

Key findings and future recommendations

Genetics and mapping

Key findings

- Australian and New Zealand *F. auricularia* populations consist entirely of subspecies B populations meaning they produce two generations per year

- Multiple introductions of *F. auricularia* have occurred on the Australian mainland, however, single introductions may have occurred in Tasmania and New Zealand
- The origin of one clade of subspecies B found only on the Australian mainland could not be determined

Recommendations

- Perform climate modelling of *F. auricularia*'s potential Australian range to determine its likely distribution in Western Australia
- Survey the levels of other invertebrate fauna in differing agro-ecosystems in Western Australia pre and post earwig invasion to better understand the impact of earwig introduction into these systems.
- Perform chemical analysis of the cuticular HCs from both subspecies of *F. auricularia* to determine whether differences exist in both their cuticular HCs and aggregation pheromones

Pheromone

Key findings

- *F. auricularia* utilise unsaturated cuticular HCs (*Z*)-9-tricosene, (*Z*)-7-tricosene, (*Z*)-9-pentacosene, (*Z*)-7-pentacosene to mediate aggregations
- Inconsistent attraction was observed to the synthetic blends tested
- The cuticular HCs were shown to vary over time in adults with a decline in unsaturated HCs correlating with the observed decline in earwig trap catches in the field

Recommendations

- Determine whether the addition of the alkadienes (*Z*,*Z*)-6,9-C_{25} (*Z*,*Z*)-6,9-C_{27} will improve ability of the four component synthetic pheromone blend to both attract more earwigs and improve the consistency of trap catches
- Manipulate juvenile hormone titres to examine endocrine control of HC production and aggregation behaviour in *F. auricularia*

Apples

Key findings

- Intraguild compatibility between earwigs and *A. mali* can successfully control WAA without targeted insecticide applications being required

- Early season earwig and *A. mali* play a key role in the control of WAA in apple orchards
- Limited evidence of intraguild predation of *A. mali* by *F. auricularia* was observed

Recommendations

- Promote higher earwig numbers in apple orchards, especially early in the season using methodologies such maintaining moderate levels of ground cover and minimising the use of broad-spectrum insecticides
- Field validate the earwig and *A. mali* estimates for WAA control across a range of climates

Cherries

Key findings

- First empirical study which demonstrates earwig damage to cherries
- The need for earwig control in sweet cherries is cultivar and bunch size dependent
- Tree age and ground cover management can impact on earwig monitoring efforts
- Other currently unidentified factors appear to impact on the level of damage experienced in differing cherry cultivars

Recommendations

- Reduce insecticide use in cherry cultivars such as Lapin where damage has been shown to be low and unrelated to earwig abundance
- Conduct damage assessments in a range of commercial cherry cultivars to determine their susceptibility to damage by earwigs
- Develop a earwig monitoring methodology that accurately estimates earwig population size irrelevant of tree age
- Monitor earwig population dynamics in cherry orchards to develop predictive spray thresholds for the cultivar's most susceptible to earwig damage
- Determine the factors that underlie the observed differences in earwig damage between cultivars including phenolic, carbohydrate and organic acid content

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Appendix

Appendix 1a. Mean (±SEM) male earwigs per trap per tree and mean (±SEM) treatment effect (treatment – hexane control) to hydrocarbons after a 12 hour period in field based experiments. Positive numbers indicate attraction. Negative numbers indicate repellency. Compounds were tested within apple and cherry trees (n = 20) in a paired design against hexane controls. Bold type indicates significant difference Wilcoxon sign rank < 0.05.

		Field experiment date								
Compound	**Concentration**	**6th Jan 2012**	**19th Jan**	**28th Jan**	**3rd Feb**	**23rd Feb**	**2nd Dec**	**7th Dec**	**22nd Dec**	**6th Feb 2013**
Mean males/trap		0.34 (0.07)	0.23 (0.06)	0.67 (0.11)	2.60 (0.33)	1.13 (0.14)	0.00 (0.00)	0.74 (0.13)		
Male wash	10 IE[#]	-0.25 (0.23)	0.05 (0.15)							
Female wash	10 IE[#]		-0.15 (0.21)							
***n*-alkane blend[a]**	0.1mg	**0.40 (0.15)**								
HC blend 2[b]	0.1mg	0.00 (0.18)								
(*Z*)-9-C_{23}	0.1mg			0.30 (0.26)						
(*Z*)-7-C_{23}	0.1mg			-0.10 (0.22)						
(*Z*)-9-C_{25}	0.1mg			0.10 (0.29)						
(*Z*)-7-C_{25}	0.1mg			-0.20 (0.32)						
alkene blend 3[c]	0.05mg				-0.15 (0.38)		0.00 (0.00)	-0.05 (0.20)	-0.05 (0.05)	-0.05 (0.20)
alkene blend 3[c]	0.1mg	0.15 (0.17)	0.3 (0.21)	0.35 (0.38)	-0.05 (0.48)			0.25 (0.43)	0.00 (0.00)	0.25 (0.44)
alkene blend 3[c]	0.2mg		0.1 (0.12)	-0.30 (0.44)				-0.50 (0.39)	-0.01 (0.07)	-0.50 (0.39)
alkene blend 4[d]	0.05mg					0.35 (0.41)	0.00 (0.00)			
alkene blend 4[d]	0.1mg				**1.55 (0.85)**	0.15 (0.32)		-0.50 (0.22)	0.05 (0.05)	-0.5 (0.22)
alkene blend 4[d]	0.2mg					-0.15 (0.35)				
alkene blend 5[e]	0.1mg					0.65 (0.37)				
alkene blend 5[e]	0.2mg					0.20 (0.28)				

[#]IE = Insect Equivalents, [*] 1 hour hexane extraction, [^] 3 x 100 µL cuticular hexane wash

[a] *n*-alkane blend; *n*-C_{21} : *n*-C_{23} : *n*-C_{25}; Blend ratio: 40:85:70

[b] Seven component blend *n*- C_{21} ; (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : *n*- C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25} : *n*- C_{25}; Blend ratio: 40: 70:20:85:80:15:70

[c] Four component blend (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25}; Blend ratio: 60:15:100:25

[d] Two component blend (*Z*)-9-C_{23} : (*Z*)-9-C_{25}; Blend ratio: 30:70

[e] Two component blend (*Z*)-7-C_{23} : (*Z*)-7-C_{25}; Blend ratio: 30:70

Appendix 1b. Mean (±SEM) female earwigs per trap per tree and mean (±SEM) treatment effect (treatment – hexane control) to hydrocarbons after a 12 hour period in field based experiments. Positive numbers indicate attraction. Negative numbers indicate repellency. Compounds were tested within apple and cherry trees (n = 20) in a paired design against hexane controls. Bold type indicates significant difference Wilcoxon sign rank < 0.05.

		Field experiment date								
Compound	**Concentration**	**6th Jan 2012**	**19th Jan**	**28th Jan**	**3rd Feb**	**23rd Feb**	**2nd Dec**	**7th Dec**	**22nd Dec**	**6th Feb 2013**
Mean females/trap		0.53 (0.09)	0.36 (0.07)	1.08 (0.16)	0.53 (0.12)	1.65 (0.19)	0.03 (0.03)	1.13 (0.18)		
Male wash	10 IE[#]	-0.35 (0.20)	0.10 (0.19)							
Female wash	10 IE[#]		-0.20 (0.17)							
***n*-alkane blend[a]**	0.1mg	-0.20 (0.16)								
HC blend 2[b]	0.1mg	0.35 (0.32)								
(*Z*)-9-C_{23}	0.1mg			0.00 (0.39)						
(*Z*)-7-C_{23}	0.1mg			-0.09 (0.55)						
(*Z*)-9-C_{25}	0.1mg			0.35 (0.53)						
(*Z*)-7-C_{25}	0.1mg			-0.50 (0.48)						
alkene blend 3[c]	0.05mg				0.60 (0.38)		0.00 (0.00)	0.15 (0.23)	0.00 (0.00)	0.15 (0.23)
alkene blend 3[c]	0.1mg	0.50 (0.30)	-0.10 (0.24)	0.90 (0.52)	0.55 (0.78)			-0.65 (0.44)	0.05 (0.05)	-0.65 (0.43)
alkene blend 3[c]	0.2mg		0.05 (0.17)	0.25 (1.12)				-0.15 (0.64)	0.15 (0.08)	-0.15 (0.64)
alkene blend 4[d]	0.05mg					0.70 (0.44)	0.01 (0.07)			
alkene blend 4[d]	0.1mg				**0.55 (0.60)**	-0.15 (0.50)		0.15 (0.45)	0.05 (0.05)	0.15 (0.43)
alkene blend 4[d]	0.2mg					0.55 (0.65)				
alkene blend 5[e]	0.1mg					1.05 (0.41)				
alkene blend 5[e]	0.2mg					0.00 (0.29)				

[#]IE = Insect Equivalents, [*] 1 hour hexane extraction, [^] 3 x 100 µL cuticular hexane wash

[a] *n*-alkane blend; *n*-C_{21} : *n*-C_{23} : *n*-C_{25}; Blend ratio: 40:85:70

[b] Seven component blend *n*- C_{21} ; (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : *n*- C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25} : *n*- C_{25}; Blend ratio: 40: 70:20:85:80:15:70

[c] Four component blend (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25}; Blend ratio: 60:15:100:25

[d] Two component blend (*Z*)-9-C_{23} : (*Z*)-9-C_{25}; Blend ratio: 30:70

[e] Two component blend (*Z*)-7-C_{23} : (*Z*)-7-C_{25}; Blend ratio: 30:70

Appendix 1c. Mean (±SEM) juvenile earwigs (2nd, 3rd and 4th instars) per trap per tree and mean (±SEM) treatment effect (treatment – hexane control) to hydrocarbons after a 12 hour period in field based experiments. Positive numbers indicate attraction. Negative numbers indicate repellency. Compounds were tested within apple and cherry trees (n = 20) in a paired design against hexane controls. Bold type indicates significant difference Wilcoxon sign rank < 0.05.

		Field experiment date								
Compound	**Concentration**	**6th Jan 2012**	**19th Jan**	**28th Jan**	**3rd Feb**	**23rd Feb**	**2nd Dec**	**7th Dec**	**22nd Dec**	**6th Feb 2013**
Mean juveniles/trap		0.52 (0.10)	0.35 (0.09)	0.76 (0.14)	2.53 (0.57)	0.04 (0.02)	8.91 (1.48)	0.49 (0.10)		
Male wash	10 IE#	0.23 (0.33)	**0.25 (0.33)**							
Female wash	10 IE#		-0.10 (0.19)							
***n*-alkane blend**[a]	0.1mg	-0.03 (0.36)								
HC blend 2[b]	0.1mg	-0.05 (0.42)								
(*Z*)-9-C_{23}	0.1mg			-2.40 (0.12)						
(*Z*)-7-C_{23}	0.1mg			-3.20 (0.30)						
(*Z*)-9-C_{25}	0.1mg			-2.90 (0.15)						
(*Z*)-7-C_{25}	0.1mg			-3.80 (0.20)						
alkene blend 3[c]	0.05mg				0.15 (0.16)		**5.63 (3.98)**	-0.23 (0.28)	-1.18 (2.76)	-0.23 (0.28)
alkene blend 3[c]	0.1mg	0.30 (0.41)	0.20 (0.30)	-3.55 (0.18)	0.23 (0.26)			**0.18 (0.26)**	-1.93 (3.34)	0.18 (0.26)
alkene blend 3[c]	0.2mg		0.48 (0.33)	-2.45 (0.12)				**-0.03 (0.45)**	0.90 (3.65)	-0.03 (0.45)
alkene blend 4[d]	0.05mg					0.03 (0.05)	1.38 (1.73)			
alkene blend 4[d]	0.1mg				-0.08 (0.27)	-0.03 (0.14)		0.15 (0.29)	1.40 (3.45)	0.15 (0.29)
alkene blend 4[d]	0.2mg					0.00 (0.00)				
alkene blend 5[e]	0.1mg					0.00 (0.10)				
alkene blend 5[e]	0.2mg					0.05 (0.10)				

#IE = Insect Equivalents, * 1 hour hexane extraction, ^ 3 x 100 µL cuticular hexane wash

[a] *n*-alkane blend; *n*-C_{21} : *n*-C_{23} : *n*-C_{25}; Blend ratio: 40:85:70

[b] Seven component blend *n*- C_{21} ; (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : *n*- C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25} : *n*- C_{25}; Blend ratio: 40: 70:20:85:80:15:70

[c] Four component blend (*Z*)-9-C_{23} : (*Z*)-7-C_{23} : (*Z*)-9-C_{25} : (*Z*)-7-C_{25}; Blend ratio: 60:15:100:25

[d] Two component blend (*Z*)-9-C_{23} : (*Z*)-9-C_{25}; Blend ratio: 30:70

[e] Two component blend (*Z*)-7-C_{23} : (*Z*)-7-C_{25}; Blend ratio: 30:70

Appendix 2. Mean (SEM) cuticular HC composition (µg of *n*-docosane equivalents) of aggregating male (n = 6) and female (n = 6) *F. auricularia* collected within an apple trees every two weeks from 16th December 2011 to 20th April 2012 and from subterranean nests on the 9th May 2012. Statistics were performed using Kruskal-Wallis test.

Collection Date	Sex	*n*-C_{21}	(*Z*)-9-C_{23}	(*Z*)-7-C_{23}	*n*-C_{23}	7Me-C_{23}	5Me-C_{23}	3Me-C_{23}	*n*-C_{24}	3,7diMe-C_{23}	6,9-C_{25}
16/12/2011	Male	0.025 (0.025)	0.511 (0.032)	0.088 (0.009)	2.184 (0.387)	0.005 (0.002)	0.018 (0.005)	0.055 (0.008)	0.054 (0.020)	0.024 (0.016)	0.221 (0.221)
30/12/2011	Male	0.075 (0.022)	0.537 (0.180)	0.313 (0.108)	0.813 (0.286)	0.017 (0.009)	0.020 (0.014)	0.074 (0.035)	0.055 (0.022)	0.014 (0.001)	0.033 (0.025)
18/01/2012	Male	0.018 (0.009)	0.064 (0.011)	0.036 (0.008)	0.307 (0.075)	0.002 (0.001)	0.002 (0.001)	0.013 (0.004)	0.036 (0.006)	0.000 (0.000)	0.010 (0.004)
6/02/2012	Male	0.033 (0.023)	0.083 (0.036)	0.069 (0.016)	0.361 (0.090)	0.001 (0.001)	0.003 (0.002)	0.009 (0.001)	0.025 (0.003)	0.000 (0.000)	0.023 (0.007)
24/02/2012	Male	0.005 (0.002)	0.086 (0.022)	0.049 (0.012)	0.225 (0.024)	0.002 (0.001)	0.001 (0.001)	0.009 (0.002)	0.011 (0.003)	0.007 (0.005)	0.018 (0.008)
9/03/2012	Male	0.015 (0.010)	0.067 (0.022)	0.047 (0.012)	0.164 (0.033)	0.001 (0.001)	0.003 (0.002)	0.008 (0.002)	0.019 (0.003)	0.003 (0.001)	0.007 (0.004)
23/03/2012	Male	0.018 (0.001)	0.043 (0.023)	0.028 (0.010)	0.125 (0.028)	0.000 (0.000)	0.003 (0.003)	0.003 (0.002)	0.020 (0.003)	0.026 (0.007)	0.008 (0.003)
6/04/2012	Male	0.016 (0.002)	0.000 (0.000)	0.004 (0.004)	0.141 (0.018)	0.002 (0.002)	0.000 (0.000)	0.002 (0.001)	0.020 (0.001)	0.027 (0.005)	0.000 (0.000)
20/04/2012	Male	0.011 (0.001)	0.039 (0.018)	0.030 (0.010)	0.229 (0.026)	0.001 (0.000)	0.004 (0.002)	0.012 (0.002)	0.035 (0.011)	0.000 (0.000)	0.003 (0.001)
9/05/2012	Male	0.004 (0.004)	0.009 (0.003)	0.002 (0.002)	0.304 (0.057)	0.000 (0.000)	0.000 (0.000)	0.001 (0.000)	0.027 (0.010)	0.005 (0.004)	0.000 (0.000)
	χ^2	***23.84***	***36.63***	***36.05***	***31.68***	***21.18***	***21.89***	***41.98***	***23.42***	***27.95***	***23.06***
	P value	***0.005***	***< 0.001***	***< 0.001***	***< 0.001***	***0.012***	***0.009***	***< 0.001***	***0.005***	***0.001***	***0.006***
16/12/2011	Female	0.018 (0.008)	0.470 (0.086)	0.310 (0.069)	1.012 (0.149)	0.025 (0.006)	0.033 (0.009)	0.103 (0.025)	0.028 (0.005)	0.031 (0.008)	0.003 (0.002)
30/12/2011	Female	0.097 (0.046)	1.608 (1.230)	0.929 (0.731)	1.313 (0.680)	0.115 (0.099)	0.125 (0.107)	0.440 (0.357)	0.206 (0.160)	0.133 (0.111)	0.079 (0.067)
18/01/2012	Female	0.020 (0.004)	0.078 (0.014)	0.065 (0.027)	0.256 (0.023)	0.001 (0.001)	0.001 (0.001)	0.009 (0.002)	0.027 (0.004)	0.002 (0.001)	0.026 (0.017)
6/02/2012	Female	0.005 (0.001)	0.045 (0.008)	0.031 (0.005)	0.178 (0.024)	0.005 (0.001)	0.004 (0.001)	0.017 (0.002)	0.015 (0.001)	0.014 (0.003)	0.023 (0.005)
24/02/2012	Female	0.002 (0.001)	0.108 (0.069)	0.070 (0.041)	0.142 (0.038)	0.014 (0.005)	0.004 (0.001)	0.029 (0.007)	0.016 (0.001)	0.047 (0.028)	0.022 (0.014)
9/03/2012	Female	0.006 (0.001)	0.072 (0.039)	0.064 (0.028)	0.105 (0.025)	0.006 (0.003)	0.003 (0.002)	0.024 (0.004)	0.014 (0.002)	0.008 (0.002)	0.085 (0.051)
23/03/2012	Female	0.013 (0.003)	0.057 (0.024)	0.035 (0.013)	0.079 (0.007)	0.009 (0.001)	0.014 (0.003)	0.037 (0.004)	0.015 (0.001)	0.040 (0.008)	0.008 (0.003)
6/04/2012	Female	0.013 (0.001)	0.014 (0.011)	0.006 (0.003)	0.101 (0.034)	0.005 (0.003)	0.010 (0.003)	0.017 (0.005)	0.013 (0.002)	0.020 (0.011)	0.000 (0.000)
20/04/2012	Female	0.052 (0.025)	0.348 (0.308)	0.106 (0.082)	0.470 (0.346)	0.029 (0.015)	0.014 (0.081)	0.124 (0.081)	0.048 (0.028)	0.068 (0.035)	0.058 (0.032)
9/05/2012	Female	0.008 (0.008)	0.174 (0.106)	0.054 (0.028)	0.398 (0.144)	0.040 (0.016)	0.063 (0.042)	0.174 (0.056)	0.066 (0.021)	0.055 (0.041)	0.007 (0.007)
	χ^2	***31.75***	***27.14***	***26.97***	***32.07***	***24.83***	***27.66***	***34.13***	***19.63***	***19.83***	***21.90***
	P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***0.003***	***< 0.001***	***< 0.001***	***0.020***	***0.019***	***0.009***

Appendix 2 cont…

Collection Date	Sex	(*Z*,*Z*)-6,9-C_{25}	(*Z*)-9-C_{25}	3,6,9-C_{25}	(*Z*)-7-C_{25}	*n*-C_{25}	13Me-C_{25}	11Me-C_{25}	9Me-C_{25}	7Me-C_{25}	5Me-C_{25}
16/12/2011	Male	1.203 (0.290)	1.139 (0.182)	0.174 (0.055)	0.126 (0.025)	1.710 (0.199)	0.071 (0.009)	0.091 (0.010)	0.062 (0.012)	0.257 (0.049)	0.026 (0.009)
30/12/2011	Male	0.396 (0.154)	2.210 (0.808)	0.023 (0.010)	1.264 (0.368)	0.855 (0.369)	0.076 (0.039)	0.087 (0.041)	0.026 (0.010)	0.581 (0.285)	0.054 (0.024)
18/01/2012	Male	0.073 (0.017)	0.453 (0.136)	0.000 (0.000)	0.141 (0.025)	0.842 (0.101)	0.021 (0.004)	0.012 (0.004)	0.002 (0.001)	0.088 (0.029)	0.007 (0.004)
6/02/2012	Male	0.049 (0.013)	0.457 (0.111)	0.000 (0.000)	0.267 (0.062)	0.723 (0.129)	0.017 (0.003)	0.017 (0.002)	0.008 (0.001)	0.090 (0.014)	0.016 (0.003)
24/02/2012	Male	0.061 (0.011)	0.441 (0.073)	0.003 (0.001)	0.127 (0.031)	0.759 (0.079)	0.018 (0.003)	0.021 (0.003)	0.006 (0.001)	0.078 (0.014)	0.010 (0.003)
9/03/2012	Male	0.080 (0.026)	0.221 (0.052)	0.007 (0.002)	0.091 (0.020)	0.441 (0.047)	0.009 (0.002)	0.012 (0.003)	0.005 (0.001)	0.026 (0.005)	0.005 (0.002)
23/03/2012	Male	0.030 (0.006)	0.142 (0.035)	0.004 (0.001)	0.059 (0.015)	0.413 (0.065)	0.014 (0.004)	0.016 (0.002)	0.007 (0.001)	0.030 (0.008)	0.001 (0.001)
6/04/2012	Male	0.028 (0.009)	0.058 (0.013)	0.003 (0.002)	0.005 (0.003)	0.480 (0.045)	0.004 (0.001)	0.008 (0.001)	0.006 (0.002)	0.010 (0.002)	0.000 (0.000)
20/04/2012	Male	0.163 (0.042)	0.521 (0.085)	0.002 (0.001)	0.122 (0.027)	0.893 (0.167)	0.023 (0.002)	0.036 (0.005)	0.015 (0.003)	0.055 (0.010)	0.022 (0.003)
9/05/2012	Male	0.042 (0.013)	0.050 (0.017)	0.003 (0.001)	0.009 (0.005)	0.561 (0.095)	0.013 (0.008)	0.009 (0.004)	0.004 (0.001)	0.004 (0.001)	0.001 (0.001)
	χ^2	***32.56***	***42.61***	***30.49***	***41.31***	***26.25***	***32.94***	***39.84***	***37.92***	***48.51***	***38.66***
	P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***0.002***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***
16/12/2011	Female	0.277 (0.080)	4.435 (0.979)	-	1.664 (0.498)	0.885 (0.081)	0.093 (0.022)	0.119 (0.030)	0.060 (0.019)	0.811 (0.192)	0.099 (0.033)
30/12/2011	Female	1.024 (0.778)	5.785 (4.189)	-	3.763 (2.855)	2.154 (1.412)	0.383 (0.317)	0.553 (0.482)	0.147 (0.119)	2.128 (1.561)	0.291 (0.248)
18/01/2012	Female	0.072 (0.031)	1.744 (1.414)	-	0.538 (0.385)	0.578 (0.098)	0.015 (0.002)	0.019 (0.003)	0.006 (0.002)	0.080 (0.023)	0.016 (0.003)
6/02/2012	Female	0.038 (0.010)	0.619 (0.203)	-	0.233 (0.040)	0.451 (0.033)	0.020 (0.004)	0.028 (0.004)	0.013 (0.003)	0.136 (0.021)	0.021 (0.004)
24/02/2012	Female	0.072 (0.016)	0.525 (0.189)	-	0.278 (0.090)	0.683 (0.036)	0.036 (0.008)	0.061 (0.026)	0.022 (0.011)	0.206 (0.054)	0.033 (0.015)
9/03/2012	Female	0.108 (0.043)	0.395 (0.175)	-	0.219 (0.096)	0.371 (0.049)	0.033 (0.005)	0.035 (0.007)	0.012 (0.001)	0.114 (0.033)	0.015 (0.002)
23/03/2012	Female	0.127 (0.052)	0.222 (0.084)	-	0.182 (0.087)	0.446 (0.023)	0.062 (0.013)	0.100 (0.017)	0.028 (0.008)	0.172 (0.033)	0.013 (0.003)
6/04/2012	Female	0.014 (0.009)	0.163 (0.081)	-	0.019 (0.009)	0.507 (0.066)	0.060 (0.022)	0.071 (0.023)	0.019 (0.004)	0.077 (0.021)	0.018 (0.003)
20/04/2012	Female	0.215 (0.179)	0.900 (0.736)	-	0.213 (0.155)	2.017 (1.212)	0.197 (0.098)	0.280 (0.140)	0.067 (0.030)	0.585 (0.372)	0.165 (0.104)
9/05/2012	Female	0.127 (0.058)	0.506 (0.182)	-	0.314 (0.131)	2.714 (0.763)	0.268 (0.100)	0.436 (0.152)	0.132 (0.038)	0.348 (0.133)	0.195 (0.062)
	χ^2	***24.41***	***26.28***		***31.56***	***28.20***	***25.10***	***28.20***	***29.89***	***24.94***	***29.12***
	P value	***0.004***	***0.002***		***< 0.001***	***< 0.001***	***0.001***	***0.003***	***0.003***	***< 0.001***	***0.001***

Appendix 2 cont…

Collection Date	Sex	3Me-C_{25}	*n*-C_{26}	3,7-diMe-C_{25}	(*Z*,*Z*)-6,9-C_{27}	(*Z*)-9-C_{27}	3,6,9-C_{27}	(*Z*)-7-C_{27}	*n*-C27	13Me-15Me-C_{27}	11Me-C_{27}
16/12/2011	Male	0.626 (0.092)	0.079 (0.009)	0.011 (0.005)	3.859 (0.631)	0.419 (0.097)	0.544 (0.070)	0.016 (0.002)	0.746 (0.108)	0.489 (0.068)	0.768 (0.104)
30/12/2011	Male	0.554 (0.278)	0.049 (0.015)	0.024 (0.012)	0.194 (0.130)	0.212 (0.154)	0.025 (0.019)	0.145 (0.082)	0.313 (0.094)	0.220 (0.090)	0.240 (0.100)
18/01/2012	Male	0.159 (0.024)	0.058 (0.009)	0.004 (0.004)	0.029 (0.007)	0.032 (0.005)	0.000 (0.000)	0.035 (0.008)	0.683 (0.170)	0.203 (0.042)	0.217 (0.045)
6/02/2012	Male	0.130 (0.016)	0.031 (0.007)	0.005 (0.002)	0.008 (0.004)	0.015 (0.004)	0.000 (0.000)	0.004 (0.002)	0.593 (0.102)	0.101 (0.017)	0.125 (0.021)
24/02/2012	Male	0.086 (0.013)	0.040 (0.006)	0.004 (0.003)	0.081 (0.023)	0.017 (0.004)	0.007 (0.003)	0.003 (0.001)	0.739 (0.102)	0.160 (0.014)	0.194 (0.014)
9/03/2012	Male	0.062 (0.010)	0.017 (0.002)	0.015 (0.003)	0.053 (0.012)	0.010 (0.005)	0.009 (0.002)	0.002 (0.001)	0.349 (0.058)	0.089 (0.004)	0.119 (0.010)
23/03/2012	Male	0.081 (0.020)	0.025 (0.006)	0.006 (0.003)	0.130 (0.016)	0.022 (0.008)	0.019 (0.004)	0.009 (0.005)	0.266 (0.045)	0.153 (0.015)	0.192 (0.022)
6/04/2012	Male	0.042 (0.005)	0.033 (0.008)	0.019 (0.009)	0.107 (0.030)	0.006 (0.004)	0.012 (0.004)	0.000 (0.000)	0.501 (0.091)	0.067 (0.029)	0.127 (0.037)
20/04/2012	Male	0.224 (0.013)	0.054 (0.006)	0.008 (0.007)	0.276 (0.093)	0.042 (0.017)	0.012 (0.006)	0.010 (0.004)	0.668 (0.089)	0.225 (0.039)	0.242 (0.039)
9/05/2012	Male	0.028 (0.005)	0.035 (0.006)	0.009 (0.004)	0.073 (0.019)	0.011 (0.004)	0.006 (0.002)	0.002 (0.002)	0.452 (0.050)	0.201 (0.095)	0.116 (0.018)
	χ^2	***48.07***	***30.15***	***12.91***	***37.15***	***31.08***	***37.60***	***37.34***	***27.26***	***28.98***	***28.94***
	P value	***< 0.001***	***< 0.001***	***0.17***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***0.001***	***0.001***	***0.001***
16/12/2011	Female	0.716 (0.179)	0.027 (0.008)	0.041 (0.010)	0.057 (0.015)	0.068 (0.028)	-	0.024 (0.007)	0.353 (0.044)	0.181 (0.031)	0.250 (0.041)
30/12/2011	Female	2.127 (1.654)	0.177 (0.131)	0.112 (0.077)	0.157 (0.105)	0.147 (0.069)	-	0.107 (0.046)	0.694 (0.403)	0.540 (0.428)	0.792 (0.607)
18/01/2012	Female	0.130 (0.021)	0.032 (0.004)	0.004 (0.003)	0.010 (0.004)	0.034 (0.010)	-	0.345 (0.327)	0.405 (0.017)	0.125 (0.010)	0.144 (0.010)
6/02/2012	Female	0.186 (0.010)	0.021 (0.002)	0.016 (0.003)	0.008 (0.002)	0.026 (0.005)	-	0.004 (0.001)	0.411 (0.072)	0.109 (0.010)	0.158 (0.010)
24/02/2012	Female	0.205 (0.032)	0.043 (0.005)	0.036 (0.013)	0.035 (0.009)	0.025 (0.008)	-	0.008 (0.002)	0.554 (0.059)	0.178 (0.005)	0.281 (0.019)
9/03/2012	Female	0.132 (0.030)	0.023 (0.002)	0.024 (0.006)	0.019 (0.008)	0.012 (0.004)	-	0.003 (0.002)	0.363 (0.050)	0.172 (0.020)	0.210 (0.017)
23/03/2012	Female	0.299 (0.026)	0.033 (0.003)	0.048 (0.014)	0.019 (0.003)	0.007 (0.003)	-	0.000 (0.000)	0.373 (0.015)	0.254 (0.036)	0.373 (0.055)
6/04/2012	Female	0.149 (0.019)	0.033 (0.004)	0.039 (0.017)	0.007 (0.002)	0.004 (0.003)	-	0.002 (0.001)	0.318 (0.071)	0.143 (0.024)	0.206 (0.035)
20/04/2012	Female	1.299 (0.763)	0.179 (0.088)	0.156 (0.075)	0.102 (0.064)	0.018 (0.015)	-	0.001 (0.001)	1.781 (0.811)	0.622 (0.296)	1.291 (0.659)
9/05/2012	Female	1.728 (0.459)	0.297 (0.129)	0.125 (0.053)	0.054 (0.021)	0.000 (0.000)	-	0.000 (0.000)	1.720 (0.530)	1.341 (0.372)	1.493 (0.367)
	χ^2	***40.34***	***28.38***	***24.27***	***20.52***	***37.26***		***43.36***	***21.98***	***28.53***	***35.20***
	P value	***< 0.001***	***0.001***	***0.004***	***0.015***	***< 0.001***		***< 0.001***	***0.009***	***0.001***	***< 0.001***

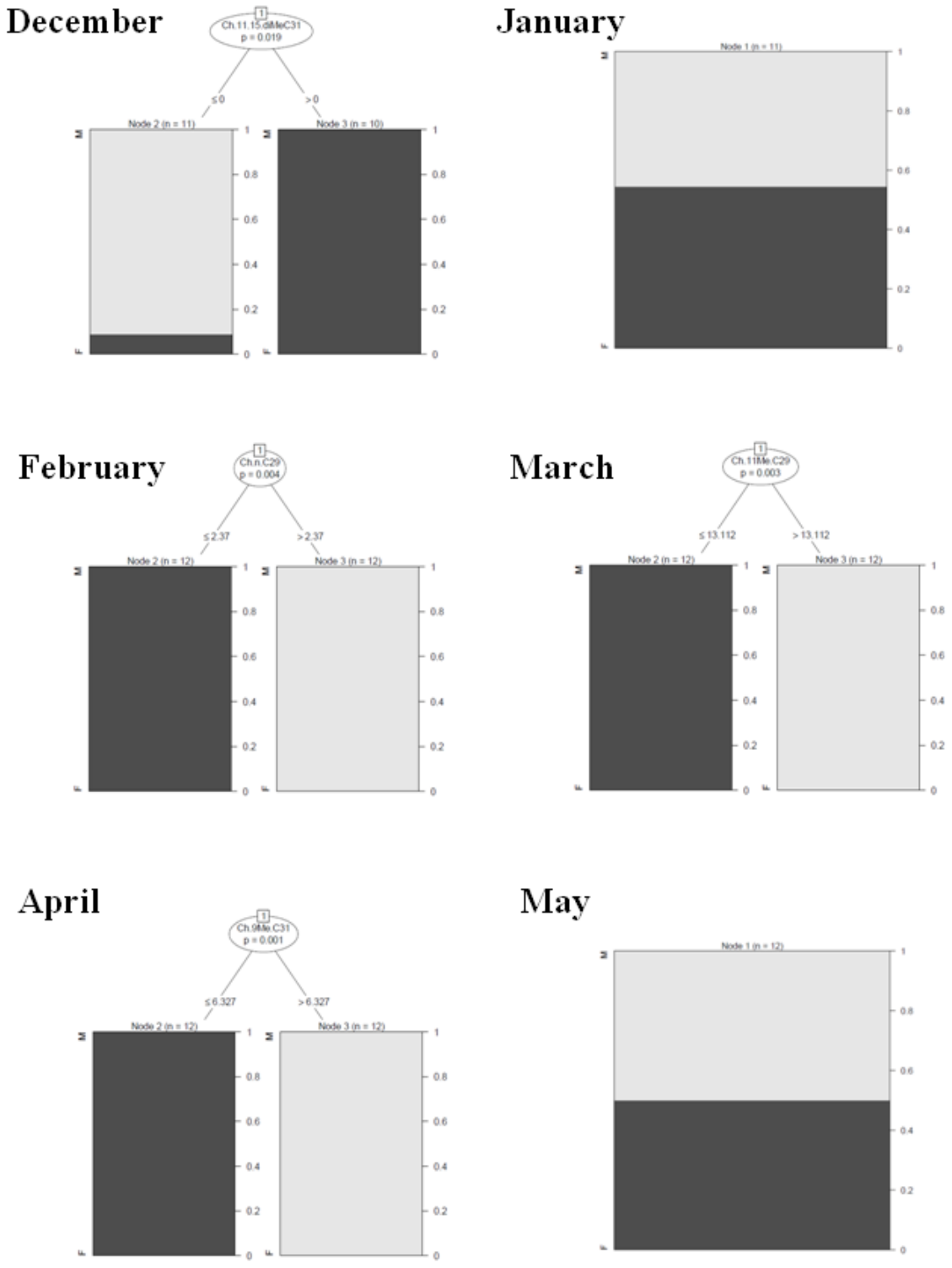
Appendix 2 cont…

Collection Date	Sex	9Me-C_{27}	7Me-C_{27}	5Me-C_{27}	11,15-diMe-C_{27}	9,13-diMe-C_{27}	3Me-C_{27}	*n*-C_{29}	15Me-C_{29}	13Me-C_{29}	11Me-C_{29}
16/12/2011	Male	1.563 (0.231)	0.579 (0.120)	0.123 (0.017)	0.139 (0.040)	0.239 (0.040)	0.873 (0.061)	0.116 (0.008)	0.304 (0.037)	0.626 (0.105)	1.485 (0.024)
30/12/2011	Male	0.552 (0.234)	0.016 (0.005)	0.041 (0.018)	0.039 (0.019)	0.119 (0.062)	0.165 (0.066)	0.030 (0.015)	0.090 (0.042)	0.151 (0.056)	0.509 (0.176)
18/01/2012	Male	0.424 (0.080)	0.011 (0.002)	0.046 (0.011)	0.018 (0.005)	0.034 (0.007)	0.164 (0.035)	0.176 (0.068)	0.181 (0.023)	0.248 (0.038)	0.672 (0.080)
6/02/2012	Male	0.222 (0.038)	0.008 (0.002)	0.030 (0.003)	0.010 (0.003)	0.017 (0.005)	0.134 (0.017)	0.094 (0.027)	0.120 (0.025)	0.157 (0.028)	0.373 (0.063)
24/02/2012	Male	0.334 (0.025)	0.039 (0.011)	0.023 (0.004)	0.029 (0.009)	0.037 (0.011)	0.140 (0.040)	0.114 (0.016)	0.193 (0.026)	0.230 (0.057)	0.621 (0.064)
9/03/2012	Male	0.203 (0.014)	0.008 (0.001)	0.019 (0.004)	0.018 (0.003)	0.014 (0.001)	0.148 (0.030)	0.067 (0.014)	0.106 (0.008)	0.191 (0.022)	0.368 (0.047)
23/03/2012	Male	0.366 (0.033)	0.019 (0.003)	0.045 (0.008)	0.035 (0.008)	0.059 (0.007)	0.272 (0.040)	0.162 (0.035)	0.230 (0.022)	0.305 (0.058)	0.671 (0.046)
6/04/2012	Male	0.249 (0.044)	0.015 (0.002)	0.032 (0.005)	0.024 (0.011)	0.056 (0.010)	0.257 (0.036)	0.137 (0.060)	0.168 (0.016)	0.301 (0.024)	0.552 (0.073)
20/04/2012	Male	0.517 (0.016)	0.010 (0.003)	0.079 (0.010)	0.048 (0.008)	0.125 (0.015)	0.379 (0.062)	0.294 (0.073)	0.253 (0.024)	0.389 (0.044)	0.918 (0.064)
9/05/2012	Male	0.163 (0.027)	0.011 (0.003)	0.017 (0.002)	0.026 (0.007)	0.032 (0.006)	0.127 (0.018)	0.043 (0.009)	0.141 (0.065)	0.282 (0.087)	0.301 (0.053)
	χ^2	***40.55***	***25.57***	***36.06***	***24.93***	***40.47***	***32.45***	***26.15***	***30.99***	***28.48***	***39.55***
	P value	***< 0.001***	***0.002***	***< 0.001***	***0.003***	***< 0.001***	***< 0.001***	***0.002***	***< 0.001***	***0.001***	***< 0.001***
16/12/2011	Female	0.529 (0.103)	0.205 (0.034)	0.049 (0.010)	0.051 (0.013)	0.059 (0.022)	0.220 (0.034)	0.021 (0.009)	0.072 (0.019)	0.143 (0.019)	0.478 (0.057)
30/12/2011	Female	1.911 (1.548)	0.056 (0.041)	0.102 (0.069)	0.071 (0.037)	0.688 (0.613)	0.510 (0.363)	0.019 (0.005)	0.192 (0.150)	0.398 (0.327)	1.465 (1.141)
18/01/2012	Female	0.283 (0.027)	0.007 (0.001)	0.024 (0.004)	0.015 (0.007)	0.030 (0.008)	0.096 (0.006)	0.075 (0.008)	0.119 (0.013)	0.138 (0.011)	0.367 (0.037)
6/02/2012	Female	0.259 (0.030)	0.010 (0.001)	0.024 (0.004)	0.014 (0.005)	0.017 (0.007)	0.095 (0.020)	0.055 (0.013)	0.090 (0.016)	0.114 (0.016)	0.254 (0.022)
24/02/2012	Female	0.418 (0.037)	0.074 (0.022)	0.028 (0.007)	0.026 (0.007)	0.049 (0.007)	0.190 (0.028)	0.086 (0.010)	0.189 (0.013)	0.259 (0.018)	0.518 (0.058)
9/03/2012	Female	0.318 (0.018)	0.008 (0.001)	0.022 (0.004)	0.033 (0.006)	0.042 (0.011)	0.164 (0.012)	0.046 (0.010)	0.124 (0.022)	0.158 (0.019)	0.331 (0.030)
23/03/2012	Female	0.637 (0.064)	0.025 (0.004)	0.057 (0.008)	0.135 (0.015)	0.171 (0.042)	0.343 (0.030)	0.114 (0.039)	0.213 (0.012)	0.267 (0.018)	0.550 (0.033)
6/04/2012	Female	0.304 (0.042)	0.011 (0.002)	0.020 (0.005)	0.046 (0.015)	0.097 (0.040)	0.101 (0.031)	0.078 (0.028)	0.082 (0.009)	0.108 (0.007)	0.213 (0.021)
20/04/2012	Female	2.092 (1.104)	0.048 (0.028)	0.282 (0.150)	0.224 (0.096)	0.631 (0.323)	0.716 (0.367)	1.027 (0.530)	0.817 (0.489)	0.985 (0.462)	1.712 (0.888)
9/05/2012	Female	2.636 (0.724)	0.105 (0.029)	0.300 (0.071)	0.468 (0.151)	0.495 (0.148)	1.391 (0.369)	0.240 (0.083)	0.728 (0.204)	1.080 (0.304)	1.888 (0.601)
	χ^2	***33.48***	***36.55***	***34.57***	***35.43***	***33.66***	***38.76***	***35.93***	***30.55***	***38.80***	***30.94***
	P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***

Appendix 2 cont…

Collection Date	Sex	9Me-C_{29}	7Me-C_{29}	11,15-diMeC_{29}	9,13-diMeC_{29}	3Me-C_{29}	15Me-C_{31}	13Me-C_{31}	11Me-C_{31}	9Me-C_{31}	11,15-diMeC_{31}	9,13-diMeC_{31}
16/12/2011	Male	1.753 (0.183)	0.442 (0.066)	1.422 (0.285)	1.358 (0.169)	0.244 (0.012)	0.357 (0.021)	0.601 (0.038)	0.937 (0.040)	1.110 (0.099)	3.238 (0.121)	1.878 (0.115)
30/12/2011	Male	0.273 (0.104)	0.061 (0.020)	0.100 (0.062)	0.174 (0.102)	0.013 (0.005)	0.067 (0.026)	0.135 (0.050)	0.260 (0.088)	0.140 (0.053)	0.123 (0.032)	0.049 (0.017)
18/01/2012	Male	0.342 (0.058)	0.189 (0.098)	0.094 (0.026)	0.094 (0.028)	0.020 (0.007)	0.116 (0.017)	0.246 (0.042)	0.344 (0.039)	0.195 (0.036)	0.174 (0.068)	0.118 (0.039)
6/02/2012	Male	0.215 (0.044)	0.057 (0.014)	0.049 (0.015)	0.050 (0.016)	0.023 (0.006)	0.092 (00.25)	0.186 (0.050)	0.305 (0.069)	0.169 (0.037)	0.216 (0.092)	0.104 (0.051)
24/02/2012	Male	0.433 (0.072)	0.135 (0.017)	0.176 (0.033)	0.093 (0.034)	0.034 (0.007)	0.211 (0.048)	0.385 (0.071)	0.488 (0.063)	0.345 (0.060)	0.624 (0.172)	0.394 (0.100)
9/03/2012	Male	0.273 (0.026)	0.054 (0.008)	0.081 (0.013)	0.087 (0.016)	0.035 (0.011)	0.092 (0.011)	0.158 (0.022)	0.195 (0.020)	0.138 (0.014)	0.375 (0.052)	0.213 (0.031)
23/03/2012	Male	0.623 (0.054)	0.116 (0.030)	0.190 (0.015)	0.172 (0.020)	0.069 (0.011)	0.256 (0.023)	0.327 (0.084)	0.502 (0.031)	0.437 (0.038)	0.688 (0.131)	0.535 (0.051)
6/04/2012	Male	0.507 (0.049)	0.117 (0.011)	0.212 (0.028)	0.203 (0.040)	0.062 (0.009)	0.205 (0.015)	0.318 (0.038)	0.317 (0.063)	0.330 (0.014)	0.885 (0.183)	0.515 (0.048)
20/04/2012	Male	0.499 (0.083)	0.164 (0.022)	0.348 (0.076)	0.150 (0.052)	0.079 (0.014)	0.201 (0.026)	0.320 (0.071)	0.495 (0.057)	0.406 (0.049)	0.632 (0.182)	0.464 (0.052)
9/05/2012	Male	0.242 (0.033)	0.061 (0.007)	0.191 (0.018)	0.153 (0.033)	0.030 (0.006)	0.111 (0.025)	0.169 (0.041)	0.180 (0.050)	0.120 (0.023)	0.603 (0.100)	0.288 (0.044)
	χ^2	***35.39***	***32.48***	***36.62***	***25.56***	***39.41***	***38.38***	***26.99***	***36.67***	***42.62***	***33.66***	***43.73***
	P value	***< 0.001***	***< 0.001***	***< 0.001***	***0.002***	***< 0.001***	***< 0.001***	***0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***
16/12/2011	Female	0.296 (0.022)	0.096 (0.014)	0.108 (0.024)	0.060 (0.025)	0.029 (0.007)	0.051 (0.011)	0.111 (0.012)	0.335 (0.031)	0.154 (0.020)	0.108 (0.013)	0.072 (0.011)
30/12/2011	Female	0.742 (0.562)	0.096 (0.072)	0.255 (0.221)	0.099 (0.034)	0.012 (0.005)	0.266 (0.226)	0.525 (0.435)	0.790 (0.597)	0.387 (0.310)	0.373 (0.312)	0.139 (0.109)
18/01/2012	Female	0.196 (0.012)	0.054 (0.009)	0.047 (0.009)	0.043 (0.011)	0.010 (0.002)	0.078 (0.014)	0.137 (0.038)	0.298 (0.022)	0.132 (0.009)	0.121 (0.025)	0.061 (0.016)
6/02/2012	Female	0.175 (0.018)	0.032 (0.008)	0.035 (0.006)	0.048 (0.015)	0.016 (0.003)	0.061 (0.010)	0.116 (0.022)	0.190 (0.035)	0.087 (0.014)	0.149 (0.049)	0.057 (0.021)
24/02/2012	Female	0.467 (0.045)	0.062 (0.014)	0.183 (0.029)	0.272 (0.037)	0.039 (0.005)	0.194 (0.020)	0.356 (0.038)	0.507 (0.041)	0.270 (0.033)	0.975 (0.114)	0.547 (0.074)
9/03/2012	Female	0.278 (0.024)	0.038 (0.010)	0.141 (0.019)	0.140 (0.030)	0.029 (0.003)	0.073 (0.011)	0.151 (0.021)	0.225 (0.043)	0.107 (0.012)	0.485 (0.041)	0.219 (0.032)
23/03/2012	Female	0.620 (0.072)	0.113 (0.017)	0.266 (0.037)	0.334 (0.035)	0.086 (0.006)	0.210 (0.011)	0.373 (0.019)	0.552 (0.029)	0.304 (0.018)	1.371 (0.070)	0.664 (0.053)
6/04/2012	Female	0.171 (0.032)	0.052 (0.007)	0.139 (0.029)	0.171 (0.043)	0.027 (0.005)	0.067 (0.009)	0.116 (0.018)	0.166 (0.022)	0.088 (0.014)	0.497 (0.089)	0.231 (0.054)
20/04/2012	Female	1.472 (0.786)	0.448 (0.226)	1.159 (0.560)	1.440 (0.777)	0.180 (0.110)	0.640 (0.381)	1.298 (0.793)	2.063 (1.070)	1.063 (0.532)	4.305 (2.183)	1.746 (1.001)
9/05/2012	Female	1.811 (0.526)	0.362 (0.097)	1.258 (0.332)	1.435 (0.361)	0.256 (0.073)	0.603 (0.177)	0.764 (0.210)	1.507 (0.432)	0.569 (0.162)	3.911 (1.422)	2.304 (0.643)
	χ^2	***37.78***	***33.31***	***41.19***	***34.55***	***36.40***	***28.53***	***24.40***	***37.18***	***34.53***	***37.97***	***37.83***
	P value	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***	***0.001***	***0.004***	***< 0.001***	***< 0.001***	***< 0.001***	***< 0.001***

Appendix 3.



Recursive partitioning conditional inference decision trees highlighting the relationship between the concentrations of adult *Forficula auricularia*'s cuticular HCs by sex and the total number of earwigs caught in earwig traps at the same time points. The number of individuals within each terminal node is denoted by the *n*-value above each box plot. The bar plots signify the proportion of each sex for each HC identified as important within the conditional inference regression tree. Single bar plots indicate no significant differences were observed between sexes for that month.